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FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI, AND FROM THE WIHURI RESEARCH INSTITUTE, HELSINKI

EFFECT OF SOME ANTITHYROID SUBSTANCES ON THE HISTOCHEMICALLY DEMONSTRABLE SUCCINIC DEHYDROGENASE ACTIVITY IN THE THYROID OF GUINEA-PIG[1]

by

A. N. KUUSISTO and ANTTI TELKKÄ

(Received for publication September 11, 1954)

In a recent report dealing with the effect of methylthiouracil treatment on the histochemically demonstrable succinic dehydrogenase activity in the organs of guinea-pig we could demonstrate that methylthiouracil caused a marked increase in the succindehydrogenase activity of the thyroid gland (1). In the present paper, this experiment is continued by testing the influence of some other drugs known to possess antithyroid and goitrogenic properties on the histochemically detectable succindehydrogenase activity of the thyroid gland.

MATERIAL AND METHODS

Forty-five male guinea-pigs weighing about 550 g were used. They were subdivided into four groups. Ten animals received daily 40 mg/kg of body weight propylthiouracil powder as an aqueous suspension by intubation into the stomach over a period of 30 days. Ten animals received by the same way sulfaguanidine suspension in a daily dose of 150 mg/kg of body weight also over 30 days, and ten received by intubation potassium thiocyanate (Merck) in a daily dose of 45 mg/kg of body weight over 30 days, too. Fifteen of their littermates served as untreated controls. The animals were

[1] Aided by a grant from the Sigrid Jusélius Foundation.

kept in identical conditions, the temperature in the room was practically constant, and they were provided with an ordinary laboratory diet *ad libitum*. After the experimental period the animals and their respective controls were killed by decapitation without any anaesthesia and bled.

For the demonstration of succindehydrogenase activity according to the method of Seligman and Rutenburg (3) the thyroids were immediately removed, weighed, frozen and sectioned simultaneously with the corresponding control with a freezing microtome at 30 μ. The frozen sections were incubated for 60 minutes in their incubation vials kept at a constant temperature of 38° C. Neotetrazolium (Montclair Research Corporation, Montclair, N. J.) was used as a histochemical indicator for succinic dehydrogenase.

RESULTS

The mean initial and final body weights of the guinea-pigs and the mean absolute and relative weights of their thyroid glands were as follows:

	Initial Body Weight (g)	Final Body Weight (g)	Weight of the Thyroid (mg)	Weight of the Thyroid (mg/10 g of Body Weight)
Controls (n=15)	575 ± 18	680 ± 21	65 ± 4	0.95 ± 0.15
Propylthiouracil group (n=10)	554 ± 22	633 ± 26	117 ± 5	1.85 ± 0.15
Sulfaguanidine group (n=10)	584 ± 24	608 ± 20	65 ± 4	1.07 ± 0.16
Potassium thiocyanate group (n=10)	511 ± 19	664 ± 24	78 ± 6	1.17 ± 0.17

The difference between the mean relative weights of the thyroid of controls and of the propylthiouracil group was statistically significant whereas the differences between the mean relative weights of the thyroid of the controls and the two other experimental groups were statistically possible only.

The thyroid glands of the control animals were scantily deposited by formazan granules indicating a weak succinic dehydrogenase activity (Fig. 1). The weak staining was concentrated in the epithelial cells. The thyroids of the propylthiouracil-treated animals

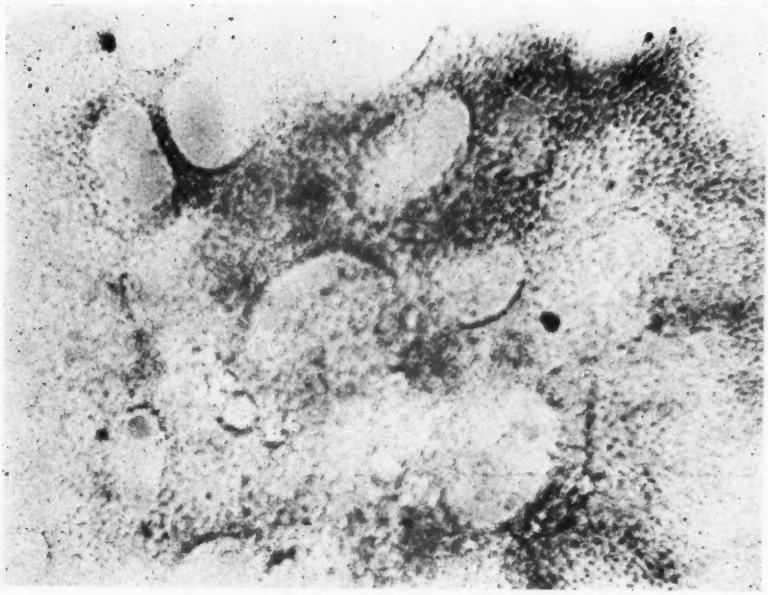

Fig. 1. — Succinic dehydrogenase activity in the thyroid of a control animal. 250 ×.

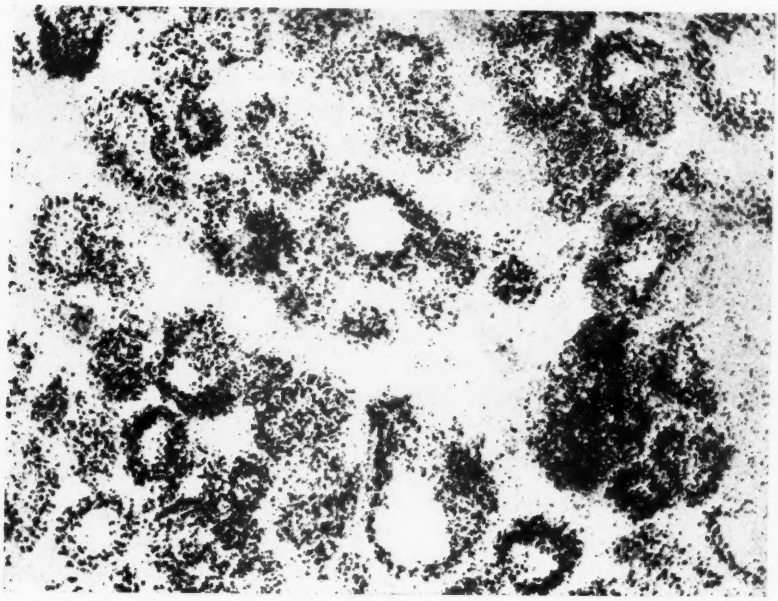

Fig. 2. — Succinic dehydrogenase activity in the thyroid of a propylthiouracil-treated animal. 250 ×.

Fig. 3. — Succinic dehydrogenase activity in the thyroid of a thiocyanate-treated animal. 250 ×.

exhibited an intense staining in the thyroid epithelium (Fig. 2). Also the thyroids of the animals which received sulfaguanidine or potassium thiocyanate revealed a clear increase in the succinic dehydrogenase activity of the thyroid epithelium as compared with the controls, but this increase was not as marked as that of the thiouracil-treated animals (Fig. 3).

DISCUSSION

All the thyroids of our experimental animals showed a marked increase of the histochemically demonstrable succindehydrogenase activity of the thyroid epithelium. This increase was most pronounced in the propylthiouracil-treated group. These results confirm our earlier observations obtained by methylthiouracil treatment (1).

The mode of action of thiourea derivatives has been explained by the fact that they cause an obstruction to the uptake of iodine into the thyroid thus preventing the completion of the synthesis of thyroid hormone (2). Thiocyanate, on the other hand, imposes an obstruction to the completion of thyroid hormone at some point

in the chain of hormone synthesis distal to the uptake of iodine (2, 4). Consequently, both these substances cause a lack of thyroid hormone which stimulates the anterior pituitary to produce an excess of thyrotrophic hormone. It can be assumed, according to our results, that this thyrotrophic stimulation is reflected on the increased oxidative metabolism of the thyroid epithelium.

In our experimental conditions and with the doses used sulfaguanidine and potassium thiocyanate resulted in only a very slight gain in the relative weights of the thyroid. When comparing this small weight gain with the clearly increased succinic dehydrogenase activity of the thyroids of these animals, it can be established that succindehydrogenase activity shows more sensitively the activity of the thyroid than the weight of this gland. Thus, it is possible that the determination of succindehydrogenase activity of the thyroid epithelium provides possibilities to improve a method for thyroid activity.

SUMMARY

Histochemically demonstrable succinic dehydrogenase activity of the thyroid of guinea-pig after administration of propylthiouracil, sulfaguanidine and potassium thiocyanate is studied. All these substances, especially propylthiouracil, resulted in a marked increase in the succindehydrogenase activity of the thyroid epithelium which was probably caused by the stimulative effect of thyrotrophic hormone.

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FROM THE WIHURI RESEARCH INSTITUTE, HELSINKI, AND THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

EFFECT OF ACTH ON SUCCINIC DEHYDROGENASE ACTIVITY IN ORGANS OF RAT[1]

A HISTOCHEMICAL STUDY

by

A. N. KUUSISTO and ANTTI TELKKÄ

(Received for publication September 29, 1954)

The metabolic effects of adrenal cortical steroids have been largely investigated experimentally during the past years. Among these studies, the succinoxidase system — an essential oxidative enzyme in the tricarboxylic acid cycle of Krebs — has been also subjected to study. According to Long (3), there is little doubt that ACTH exerts its effects on carbohydrate and protein metabolism by increasing the rate of secretion of adrenal cortical steroids. In our previous investigations on the hormonal influences on the histochemically demonstrable succindehydrogenase activity (4, 6, 7) we noted that adrenalectomy resulted in a decrease in the succindehydrogenase activity in rat tissues which could be restored by the administration of cortisone (4). The present study was carried out in order to investigate whether the administration of ACTH to intact rats influences the histochemically detectable succindehydrogenase activity.

MATERIAL AND METHODS

Adult male albino rats of Sprague-Dawley strain, weighing about 200 g, were used. Six of them received ACTH (»ACTHAR», Armour) in a daily dose of 15 mg/kg of body weight by intramuscular injection over a period of five days. Six rats received by the same manner prolonged ACTH (»Acton Prolongatum», Fred-

[1] Aided by a grant from the Sigrid Jusélius Foundation.

eriksberg chemiske Fabrikker Akts., Copenhagen) in the same dose. In addition, five rats were adrenalectomized unilaterally three weeks before sacrifice in order to investigate whether the stimulation of the remaining adrenal by this way influences its histochemically detectable succinic dehydrogenase activity. Seventeen of the littermates of the experimental rats served as untreated controls. The rats were provided with an ordinary mixed food and tap water *ad libitum*. After the experimental period, the test animals and their respective controls were sacrificed by decapitation without any anaesthesia and bled.

For the demonstration of the succinic dehydrogenase activity the histochemical technique of Seligman and Rutenburg (5) was followed using neotetrazolium (Pannone Chemical Company, Verona, N. J.) as an indicator for succinic dehydrogenase. The pieces from the heart, kidney, liver, skeletal muscle, pancreas, stomach and the adrenals were immediately removed, frozen and sectioned simultaneously with the corresponding control specimen with a freezing microtome at 30 μ. The frozen sections were dipped directly from the cooled knife into their incubation vials kept at a constant temperature of 38°C. The incubation time was, depending on the activity of the organ in question, 15 to 60 minutes.

RESULTS

The mean weights of the animals and the absolute and relative weights of their adrenal glands were as follows:

	Body Weight (g)	Weight of the Adrenals (mg)	Weight of the Adrenals (mg/10 g of Body Weight)
ACTH-treated rats (n=6)	202 ± 5	28.2 ± 0.8	1.40 ± 0.07
Control rats (n=6)	188 ± 6	22.2 ± 0.7	1.18 ± 0.10
Acton Prolongatum-treated rats (n=6)	196 ± 6	32.2 ± 0.8	1.64 ± 0.09
Control rats (n=6)	224 ± 4	26.0 ± 0.9	1.16 ± 0.11
Unilaterally adrenalectomized rats (n=5)	209 ± 7	15.7 ± 0.9[1]	0.75 ± 0.11[1]
Control rats (n=5)	198 ± 8	23.0 ± 0.8	1.16 ± 0.10

[1] Weight of the right adrenal only.

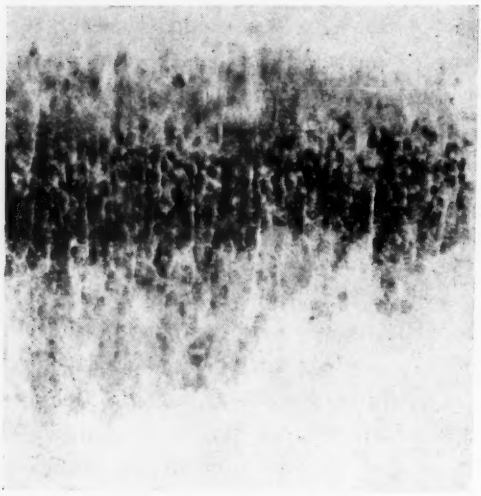

Fig. 1. — Succinic dehydrogenase activity in the gastric mucosa of a control rat. × 250.

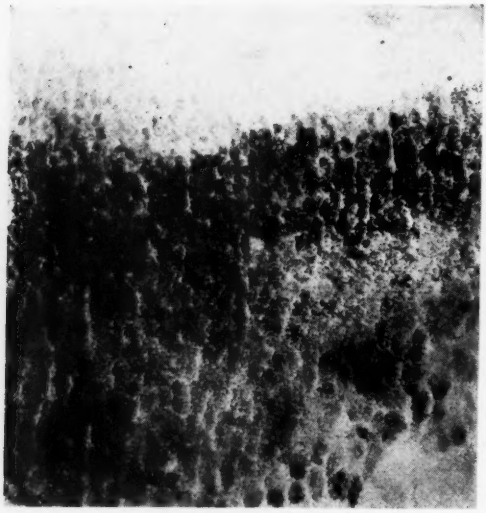

Fig. 2. — Succinic dehydrogenase activity in the gastric mucosa of an ACTH-treated rat. × 250.

The differences of the relative adrenal weights between each experimental group and their respective control groups were statistically significant.

The heart, kidney, liver, skeletal muscle and pancreas sections of the ACTH-treated animals displayed in the histochemical demonstration of succindehydrogenase an enzymatic activity substantially similar to that of the respective control sections. A slight tendency to the more intense succindehydrogenase activity — as estimated from the changes in staining intensity — could be noticed in some sections of the experimental series, but no definite differences could be observed. The weak succindehydrogenase activity of the adrenal gland, especially noticeable in the glomerular zone, was not modified by ACTH-treatment.

In contrast, the cells of the gastric mucosa of the ACTH-treated animals exhibited constantly a more pronounced succindehydrogenase activity than their respective controls (Figs. 1 and 2).

No differences could be found in the enzymatic activity in the organs of the test animals treated with ACTH or prolongated ACTH. The adrenals of the unilaterally adrenalectomized animals showed a succindehydrogenase activity similar to that of their controls.

DISCUSSION

According to our results, the treatment with ACTH did not substantially influence the histochemically demonstrable succinic dehydrogenase activity in the organs tested, except that of the gastric mucosa. In contrast, adrenalectomy results in a clear and constant reduction in the histochemically detectable succindehydrogenase activity (6) which could be restored by the administration of cortisone, but not with desoxycorticosterone (4). Thus, it seems possible that glucocorticoids play an important role in maintaining the normal succinic dehydrogenase activity in the tissues, but it does not seem to be possible to demonstrate by the histochemical method used whether the stimulation of the adrenal cortex by ACTH could cause an increase in the succindehydrogenase activity.

It has previously been shown that ACTH stimulates both the exocrine and endocrine (uropepsin) functions of gastric glands (2). It has also been found that adrenalectomy results in certain cyto-

logical alterations in the cells of the gastric mucosa of the rat which could be restored by administration of adrenocortical hormones (1). Our observations on the stimulative effect of ACTH on the succindehydrogenase activity in the cells of the gastric mucosa are in agreement with these earlier findings.

SUMMARY

The effect of ACTH on the histochemically demonstrable succinic dehydrogenase activity in the organs of rat is investigated. The enzymatic activity of the heart, kidney, liver, pancreas, skeletal muscle and adrenals was not altered after ACTH treatment, whereas ACTH resulted in a constant and clear increase in the succindehydrogenase activity of the cells of the gastric mucosa.

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FROM THE DEPARTMENTS OF MEDICAL CHEMISTRY AND OF ANATOMY,
UNIVERSITY OF HELSINKI

EFFECT OF ADRENAL STEROIDS ON SYNOVIAL TISSUES IN VITRO

by

EINO KULONEN and ANTTI TELKKÄ

(Received for publication September 6, 1954)

In connection with the studies on the cortisone action a peculiar coarse granulation was described in the nuclei of the synovial tissue cells (Kulonen, 1953). A new detailed investigation with pure steroids was prompted by the report by Schrek (1953), who found the coarse granulation in the nuclei of lymphocytes after incubation with adrenal corticoids but considered it to be unspecific.

MATERIAL AND METHODS

Knee joints were prepared immediately after killing from adult albino rats and placed into erlenmeyer flasks containing the incubation fluid (50 ml) and kept at + 37° C for 6—8 hours. A few experiments were made using about 2 g of rat skin, dissected from the backs. The incubation fluid consisted of Ringer-carbonate containing crystallised sodium penicillin G (Merck, about 200 units/ml). The steroid was added as solid and suspended with glass rod as finely as possible. The control was incubated without any added steroid.

Following preparations were used: Kendall's Compounds E and F, Reichstein's Substance S and desoxycorticosterone (DOC) as crystalline free alcohols in concentrations of 120 μg./ml, and Cortone (Merck), which reportedly contains cortisone acetate, benzylalcohol, polyoxyethylene sorbitan mono-oleate and sodium carboxymethylcellulose, in concentrations of 150 μg/ml and 1 500 μg/ml in regard to cortisone.[1])

The specimens were fixed in 80 per cent ethanol containing one per cent of trichloroacetic acid, imbedded in paraffin and sectioned at 5 μ. The

[1] For these materials we are indebted to Dr. W. W. Umbreit, Merck Institute for Therapeutic Research, Rahway, N. J., U.S.A.

stainings employed were as follows: haematoxylin-van Gieson method, silver impregnation method combined with orcein and picroaniline blue according to Lewis & Jones (1951), toluidine blue staining, periodic acid-Schiff (PAS) technique, Hale's (1946) dialysed iron method for acid mucopolysaccharides, and the histochemical methods of Bennett (1951) and of Barrnett & Seligman (1952) for the sulfhydryl groups.

RESULTS

In the synovial tissue of the controls, the most common cell types were the comma-like and ovoid cells according to the classification of Key (1932). The nuclei were mainly of the large and oval type with a granular chromatin net. The fibrous elements of the control synovial tissue stained easily with PAS technique and moderately with Hale's method. The combined silver impregnation stained the collagenous fibres lightly and some brownish-yellow fibres were also observed. When employing the method of Barrnett & Seligman for sulfhydryl groups the synovial tissue remained unstained whereas with the method of Bennett a weak yellow colour could be seen in the whole tissue. With toluidine blue stain some mast cells were noted.

The most pronounced changes in the series incubated with adrenocortical steroids were observed in the nuclei. In the samples treated with Compound E only a very few were intact, about 80 per cent were pyknotic and the others showed coarse granulation and karyolysis. These alterations were not so marked in the specimens treated with Cortone. About one half of the nuclei were intact, the remaining revealed mainly pyknotic changes. Similar changes were observed also in regard to the Compound F. The nuclei of the synovial cells incubated with Substance S were intact in about a half and pyknotic in the others. Similar changes were observed in somewhat less degree in the synovial cells incubated with DOC. The coarse granulations, instead, were rather numerous in these groups.

The mast cell granules were poorly stained in the specimens incubated with Compound E or Cortone. In the other steroid-treated groups the mast cells were not detectable. The fibrous stainings and the Hale's stain for mucopolysaccharides were not essentially influenced by the incubation with steroids and no variations were neither observed in the sulfhydryl stains.

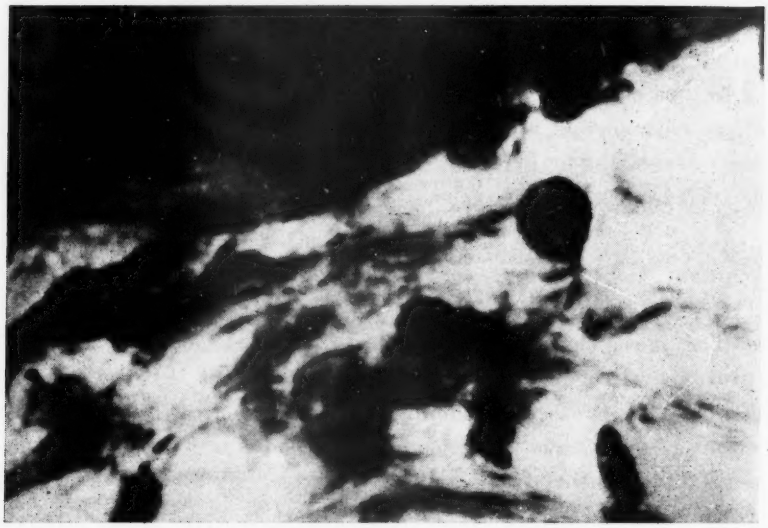

Fig. 1. — Normal synovial tissue of rat knee joint just below the patella. Large nuclei with coarse chromatin net. Haematoxylin—van Gieson. About 1100 ×.

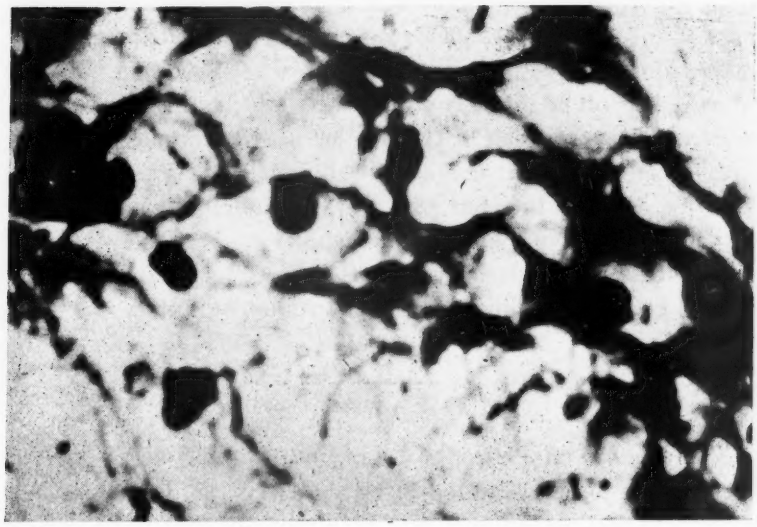

Fig. 2. — Synovial tissue incubated with Compound E. Mainly pyknotic nuclei. Haematoxylin—van Gieson. About 1100 ×.

In the skin preparations, similar pathological changes of the nuclei were observed in the cells of the stratum Malpighii and in the nuclei of the cells of the hair sheaths. The most characteristic finding was the coarse granulation whereas the pyknosis was rare. These pathological alterations were mainly present in the superficial layers, and there were also groups of granules, probably derived from karyolytic cells.

DISCUSSION

The most characteristic finding in our experiments was the cytotoxic effect of the adrenocortical steroids on the tissues. Summarising the results, the main cytological effect of Compounds E and F is pyknosis. The coarse granulations were observed in all the samples, rather frequently in those treated with DOC or Substance S.

Pyknosis is claimed to be associated with proteolysis and depolymerisation of the nucleic acids (Leuchtenberger, 1951). The amount of nucleic acid present in the mitochondria and microsoma in the rat liver was very significantly reduced with cortisone treatment (Lowe & Williams, 1953). Considering also that the migration of the fibroblasts is inhibited with cortisone (Kaufman, Mason & Kinney, 1953) it might be concluded that one phase of the cortisone effect is the suppression of the cellular activity through the nucleoproteins in the nuclei. However, Gell & Hinde (1953) claim that cortisone does not interfere with the function of the macrophages. Perhaps the cell types differ in the liability to cortisone.

The coarse granulation seems to be an unspecific effect, but whether it is purely physicochemical, cannot be stated.

SUMMARY

The main *in vitro* effect of Compounds E and F on the synovial tissue seems to be pyknosis which is less frequent in the specimens treated with Substance S or DOC. A coarse granulation was frequently observed in all groups.

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FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

EFFECT OF GIANT DOSES OF INSULIN ON THE CELLS OF THE SUPRARENAL GLAND OF RABBITS

by

ESKO K. NÄÄTÄNEN

(Received for publication September 16, 1954)

A number of scientists have been able to prove that the suprarenal gland has a central position in the effort of the organism to counteract the effect of insulin injections.

While investigating the paradoxical effect of giant intravenous insulin injections on rabbits, it seemed worth-while to try to ascertain which of the layers of cells of the suprarenal gland primarily counteracts the effect of insulin.

MATERIAL AND METHOD

Tests were performed on 25 male rabbits, each weighing about 2 kg. They were given from one to four intravenous insulin injections, the doses varying from 4 to 400 units per kg. Renewed injections were given after intervals of one week. The rabbits received their ordinary feed the whole time.

The injections were given at 1 p.m. If several injections were given, the rabbits often received an intravenous injection of glucose in the evening to check the effect of the insulin.

In order to remove the suprarenal gland, the rabbits were decapitated under a slight ether anaesthetic. The suprarenal glands were then immediately fixed in Zenker's fluid and embedded in paraffin in the usual way. In staining the sections, Delafield's haematoxylin, counter-stained with eosin, was used.

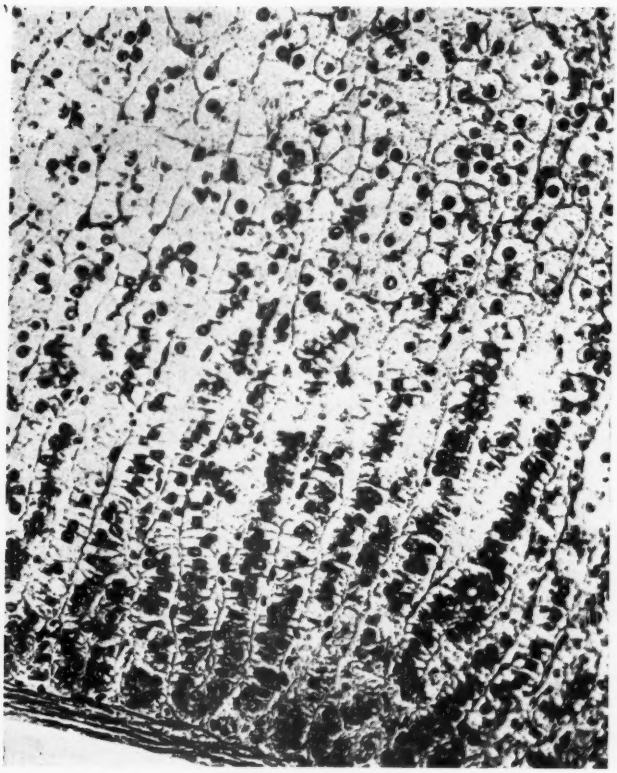

Fig. 1. — Photomicrograph showing a section of the suprarenal gland of rabbit No. 37, which received 400 units of insulin per kg and was given a renewed injection of 400 units per kg after two days. It was killed 8 hours later. × 280.

RESULTS

Several investigators have shown that insulin causes hypertrophy of the suprarenal gland. As the animals in my experiment were usually killed after the insulin had been effective only rather a short time, no distinct hypertrophy of the suprarenal gland could be noticed. Distinct changes could be observed in all the preparations, especially in the zona fasciculata. The cells were usually rather swollen; in some cytoplasm largely disappeared. Some nuclei were contracted, some again only slightly stained, as if dissolving. In some columns long strands of cells had disappeared, only the walls remaining visible. In the columns next to them, however, the cells

on the same level may not have undergone any changes at all. It would appear that the changes advance in columns.

If the effect of insulin had been of a short duration, the most distinct changes could be observed usually in the part of the zona fasciculata nearest the zona glomerulosa. If again the action of the insulin had been of a long duration and especially if the rabbit had received several insulin injections, there could be observed distinct changes throughout the area of the zona fasciculata. In these cases all the cells of the zona fasciculata were vesicularly swollen and slightly stained. The cytoplasm had often largely disappeared and in the swollen cells the nuclei had more or less undergone degenerate changes. Even the nuclei had disappeared in places (Fig. 1). In some preparations swollen cells, or cells in which the nuclei had undergone changes, could be seen also in the zona glomerulosa and the zona reticularis. The changes in the zona glomerulosa and the zona reticularis were rather slight compared with the changes in the zona fasciculata.

In one of my previous works (1) it has been shown that giant insulin doses seem to cause a marked increase in the pituitary secretion, especially from the β-cells. The heavy secretion from the pituitary in turn causes an increase in the activity of the suprarenal gland. This exceptionally strong activity results in a gradual degeneration of the cells, especially in the zona fasciculata.

SUMMARY

The effect of giant doses of insulin on the different layers of cells of the suprarenal gland of 25 male rabbits was studied.

In order to cause distinct changes, large doses of insulin were used, often as much as 400 units per kg. In all cases distinct changes could be observed in the zona fasciculata; the cells were greatly swollen and cytoplasm would disappear. There could be seen different signs of degeneration in the nuclei. Sometimes the nuclei were only slightly stained and appeared to be dissolving; sometimes again, especially if the action of the insulin had lasted for some time, there could be seen quite a large number of contracted nuclei.

When the insulin had acted only for *a short time*, changes occurred primarily in the cells of the zona fasciculata nearest the zona glomerulosa. The changes seemed to advance in columns.

When the insulin had acted for *a longer time*, especially in the event of several injections, almost all the cells in the zona fasciculata became greatly swollen and, owing to the disappearance of the cytoplasm, slightly stained. Most of the nuclei in the zona fasciculata had undergone degenerate changes of different degrees.

It was observed that some cells in the zona glomerulosa and the zona reticularis had become swollen and undergone aforedescribed degenerate changes, but changes in these layers of cells occurred comparatively seldom.

It would appear that the reaction caused by insulin greatly increases the activity of the cells in the zona fasciculata, finally resulting in the degeneration of those cells.

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FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

CYTOLOGICAL CHANGES CAUSED BY INSULIN IN THE PITUITARY OF RATS

by

ESKO K. NÄÄTÄNEN

(Received for publication September 16, 1954)

In a previous work (1) I have described the paradoxical effect of large doses of insulin. Upon its being proved that this paradoxical effect was caused by the activity of the pituitary, my next task was to ascertain what part the different cells of the pituitary play in this phenomenon.

In another publication (2) I have reported the results of an experiment performed on rabbits to determine the changes in the cells of the pituitary caused by insulin acting for a short period. In the following work I shall deal, primarily, with the changes in the pituitary of rats caused by insulin injections in experiments of longer duration.

MATERIAL AND TECHNIQUE

Tests were performed on young male rats weighing from 150 to 200 g. Some received only one subcutaneous insulin injection of 8 to 100 units; others were given several injections at intervals of about two weeks, the doses varying from 4 to 100 units. Rats which received several injections were killed after varying lengths of time; a couple of them were kept alive as long as three months after the first injection. The rats were fed normally during the period of the experiment.

The rats usually endured large insulin doses astoundingly well; some rats could stand as much as 100 units without cramps. Indi-

vidual differences are apparent. Subsequent insulin injections seem to have a stronger effect than the first one.

Usually the injections were given at 1 p.m. If several injections were given, the rats received subcutaneous injections of glucose in the evening in order to stop the effect of the insulin. After the experiment the rats were decapitated and the pituitary removed. The preparation was fixed in Zenker's fluid and embedded in the usual way. The sections, 4 μ in thickness, were stained according to Mann's long method.

RESULTS

Previous experiments have shown that small insulin injections do not cause marked changes in the cells of the pituitary body. This has also been observed by Schultze 3) who during the course of 21 days twice daily injected 0.2 units of insulin in rats. He could not notice any difference in the weight of the pituitary of the insulin-treated rats as compared with that of the control rats.

As the small doses did not seem to cause any changes in the cells of the pituitary, it was found necessary to use rather large amounts of insulin. For a detailed description of the easily recognised changes in the cells of the pituitary caused by these large insulin injections, the experiments have been divided into two groups, those of a short duration and those of a longer duration.

Some rats were killed 8 hours after the injection. The α-cells were bright orange in colour without distinct granules. The β-cells appeared to have increased in number; they were enlarged and dim. Usually no distinct granules could be seen but the cells were exceptionally strongly stained.

Some rats received several insulin injections during periods ranging from one to three months. The microscopic picture of the pituitary of these rats differs considerably from that described earlier. In experiments of a long duration marked changes in the cells of the pituitary body were found. The degree of these changes was correlative to the amount of insulin and to the length of time during which the injections were given. In these cases degenerate changes in the microscopic picture of the pituitary were characteristic. Darkly stained contracted nuclei could often be seen in abun-

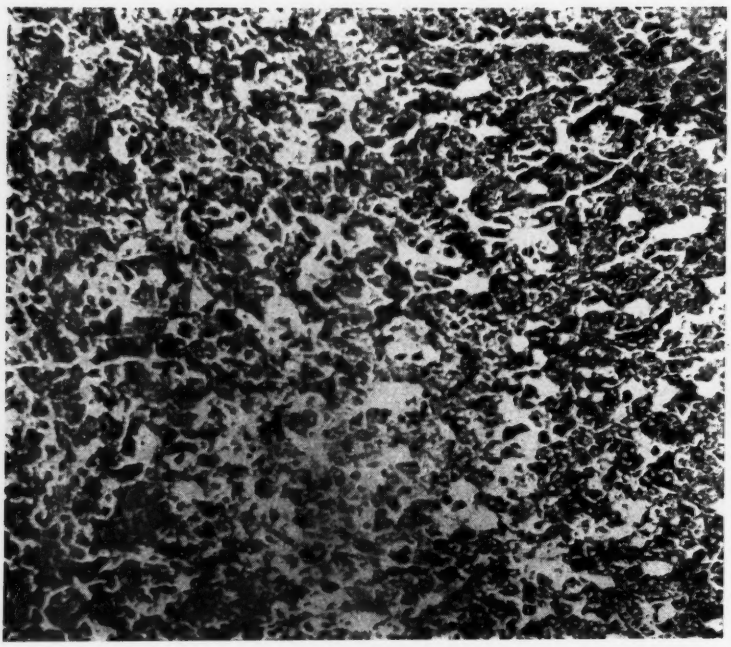

Fig. 1. — Photomicrograph showing a section of the pituitary body of rat No. 9, which was given 6 × 8 units of insulin during the course of three months. × 280.

dance. Some α-cells seemed to have undergone degenerate changes; in some cases nuclei were contracted or the cytoplasm had partly disappeared. Compared with the cases where the action of the insulin was of a shorter period, considerably less β-cells could be seen. These seemed to have undergone degenerate changes more often than the α-cells. In some preparations the dark-stained contracted nuclei were surrounded by a light ring caused apparently by the contraction. In some cells there could be seen vacuoles of different sizes (Fig. 1).

As shown in the foregoing there are marked differences in the changes caused by insulin in experiments of a short duration compared to those lasting several weeks.

A large dose of insulin acting for a short time causes changes in the cells of the pituitary, which obviously are signs of increased activity. It seems to affect especially the β-cells, which seem to increase in number, become enlarged and strongly stained. If the action of insulin is of a longer duration, there are marked changes

indicating degeneration of the cells, especially of the β-cells. Large doses of insulin acting for a longer period cause damage also to the α-cells, although in a lesser degree.

SUMMARY

In order to ascertain which group of the cells of the pituitary are mostly affected by insulin, a number of young rats were given one or more subcutaneous insulin injections, the doses varying from four to one hundred units. Usually the rats endured these exceptionally large insulin injections remarkably well, although there could be noticed considerable individual differences. Subsequent injections seem to have a stronger effect than the first one.

If the rats were killed a few hours after the insulin injection, changes could be observed in the cells of the pituitary indicating increased activity. These changes could be observed remarkably well in the β-cells.

If the rats had received renewed doses of insulin during the course of several weeks, marked degenerate changes in the pituitary resulted, especially in the β-cells, though also in some cases the α-cells were affected to some extent.

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FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

EFFECT OF GIANT DOSES OF INSULIN ON VAGOTOMISED RABBITS

by

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According to Lucke and Koch (1) the left vagus-nerve plays an important part in the reaction caused by insulin in the organism. These investigators conclude that the decrease in blood sugar, upon falling under the limit of 60 mg per cent, causes secretion of the contra-insular hormone. The decrease in blood sugar causes in all probability an excitation, which, according to Lucke and Koch, proceeds from the large glands of the abdomen, along the left vagus-nerve up to the mid-brain, entering the pituitary and causing in the pars anterior the secretion of the contra-insular hormone into the cerebro-spinal fluid.

Tests were performed by the said investigators on dogs whose vagus-nerve had been severed near the diaphragm under the Pernokton-narcosis. The animals received insulin injections the day following the operation. The investigators could not observe any reaction if the left or both vagus-nerves had been cut off. If the right vagus-nerve was severed, however, according to the investigators, the operation did not hinder the reactive hormone secretion. The amount of insulin used by Lucke and Koch was rather small, usually no more than 10 units.

As shown in a previous work (2), a very strong reaction in the organism of a rabbit is produced by large intravenous insulin injections. This reaction causes the surprising phenomenon that the rabbits often seem to be able to endure giant doses of insulin better

than quite small ones. It was interesting to discover whether this phenomenon could be observed in rabbits with severed left vagus-nerve. In three of my later works (3, 4, 5) I have studied the changes in the cells of the pituitary and the suprarenal glands caused by giant doses of insulin. If the observation, made by Lucke and Koch, of the importance of the vagus-nerve in the reaction caused by insulin, proved true also in the case of rabbits, it could be expected that changes in the cells of the pituitary and the suprarenal glands caused by insulin injections could not be observed in animals in which either the left vagus-nerve or both nerves had been severed prior to the injection. On the other hand, changes in the cells of animals whose right vagus-nerve had been severed could be expected, as, according to the said investigators, the operation of the right vagus would not hinder the reaction against the insulin in the organism.

MATERIAL AND TECHNIQUE

Tests were performed on 10 male rabbits each weighing about 2 kg. Under a slight ether anaesthetic the left vagus-nerve was severed in the neck of nine rabbits and the right vagus-nerve in one. The operations were performed successfully. Some of the animals received intravenous insulin injections the following day. To eliminate the possible effect of the operation on the reaction caused by insulin, some rabbits were given the insulin injection from four to fourteen days afterwards. Insulin doses given varied from 50 to 400 units per kg. One of the rabbits was given 100 units of insulin per kg twice, with an interval of one week. Another received 50 units of insulin per kg three times with intervals of one week. The rabbit whose right vagus-nerve had been severed was given 400 units of insulin per kg.

Immediately prior to and also one, two and three hours after the insulin injection, the blood sugar was examined according to the Crecelius-Seifert method. For histological research the rabbits were decapitated and the specimens removed. After weighing, the pituitary and the suprarenal glands were fixed in Zenker's fluid and embedded in the usual way. The pituitary was mostly stained according to Mann's long method. In staining the suprarenal glands Delafield's haematoxylin-eosin was used chiefly.

RESULTS

THE EFFECT OF INSULIN ON THE GENERAL CONDITION

As revealed in previous studies there are great individual differences in the ability of rabbits to withstand giant intravenous insulin doses. The same observation was also made in respect to vagotomised rabbits. Several had cramps 1½ to 2 hours after the injection. Rabbits whose vagus-nerve had been cut off the previous day suffered cramps readily. In all probability, ether used in the operation still hindered the activity of the cells of the pituitary.

Some rabbits whose vagus-nerve had been severed could endure quite large insulin doses remarkably well. For instance, one rabbit, weighing 2 600 g, whose left vagus-nerve had been severed one week previously, seemed able to endure an injection of 1 000 units of insulin for several hours without difficulty. It was given glucose four hours after the injection to check the effect of insulin and two weeks later killed to obtain histological specimens.

It appears that the ability of vagotomised rabbits to endure renewed insulin injections deteriorates just as in previously described experiments on unoperated rabbits.

In my material there was only one rabbit whose right vagus-nerve had been cut off. It had cramps two hours after the injection and had to be killed two hours later.

THE EFFECT OF INSULIN ON BLOOD SUGAR

According to Lucke and Koch the blood sugar decreased rather quickly in dogs which had the left vagus severed. If, instead, the right vagus-nerve had been severed the decrease in blood sugar was slower.

Fig. 1 shows the decrease of blood sugar in four rabbits after the insulin injection. Rabbit No. 1, the left vagus of which had been severed, had received an intravenous insulin injection of 100 units per kg one week previously. Prior to the examination of blood sugar it was again given the same amount of insulin. The renewed injection had a strong effect; the rabbit received cramps 1½ hours after the injection and the blood sugar decreased sharply. The decrease of blood sugar was somewhat slower in rabbit No. 2, which also had received a renewed dose of insulin of 50 units per kg. Rabbit No. 4,

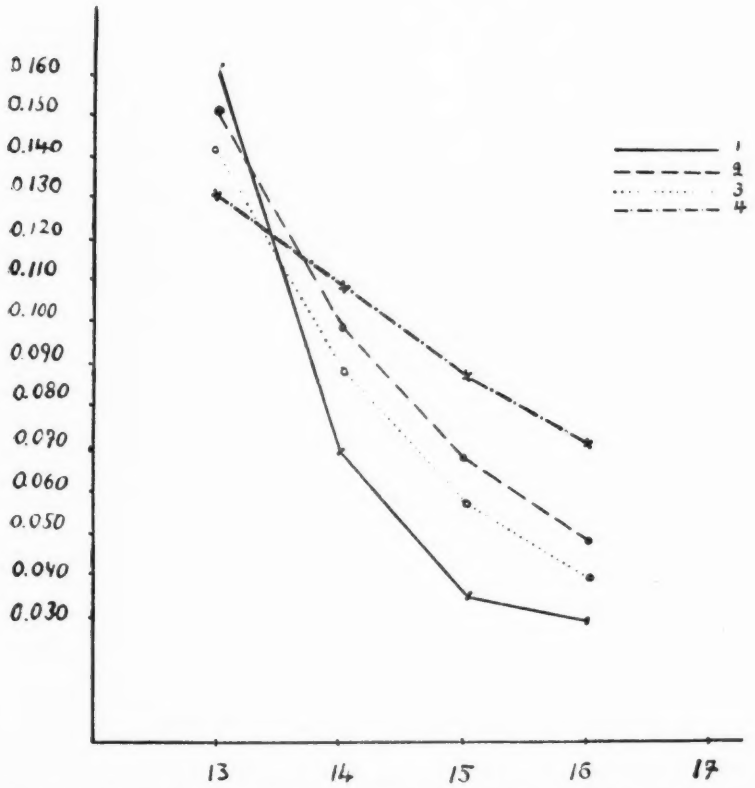

Fig. 1. — Diagram showing the decrease of blood sugar of four rabbits after the insulin injection.

which also had the left vagus severed received an insulin injection of 400 units per kg. The decrease of blood sugar was slow, much the same as in rabbits which had had no operation and which also had received large doses of insulin. The decrease in blood sugar in these, because of the reaction caused by giant insulin doses, was unexpectedly slow. Rabbit No. 3 had the right vagus-nerve severed one day previous to the insulin injection of 400 units per kg, the effect of which was a rather sharp decrease in blood sugar.

Thus my observations do not agree with those made by Lucke and Koch. According to these investigators the blood sugar curve of rabbits No. 1, 2 and 4, the left vagus of which had been severed, ought to have decreased more sharply and more rapidly than that of rabbit No. 3, the right vagus-nerve of which had been severed.

CHANGES IN THE CELLS OF THE PITUITARY BODY

In all rabbits whose left or right vagus-nerve had been severed, changes could be observed in the cells of the pituitary similar to those caused by giant insulin doses in rabbits on which the operation was not performed. The changes appeared especially in the β-cells. If the action of insulin had lasted a rather short time, the cells became swollen, exceptionally distinct granules could be seen and the cells appeared to increase in number. If, however, the action of insulin had lasted a longer time, especially if renewed injections were given, degenerate changes usually occurred. In some β-cells the cytoplasm had partially disappeared and changes in the nuclei could also be observed.

The changes in the α-cells were less easily recognised; usually no distinct granules could be observed. In some cases the α-cells also appeared to become slightly swollen.

CHANGES IN THE SUPRARENAL GLANDS

Obvious hypertrophy of the suprarenal gland appeared in some rabbits but no positive conclusions can be drawn, owing to our limited material.

Giant insulin doses caused changes in the cells of the suprarenal glands, especially in the zona fasciculata, as in the experiments on rabbits that had not had the operation on the vagus-nerve. The cells often appeared vesicularly swollen and slightly stained. Some nuclei seemed to be disappearing or had shrunk. In some columns there were strands of cells that had disappeared except for the walls (Fig. 2).

If, according to Lucke and Koch, severing the left vagus-nerve would prevent a reaction in the organism, while an operation on the right vagus-nerve had no effect on it, no changes in the cells caused by this reaction should be observed in rabbits with a severed left vagus-nerve. However, after insulin injections all the anima s u sed in the test had undergone changes in the cells of the pituitary and the suprarenal glands similar to those in animals that had not had the operation. Similar changes in the cells occurred in all the rabbits of which either the right or the left vagus-nerve had been cut off.

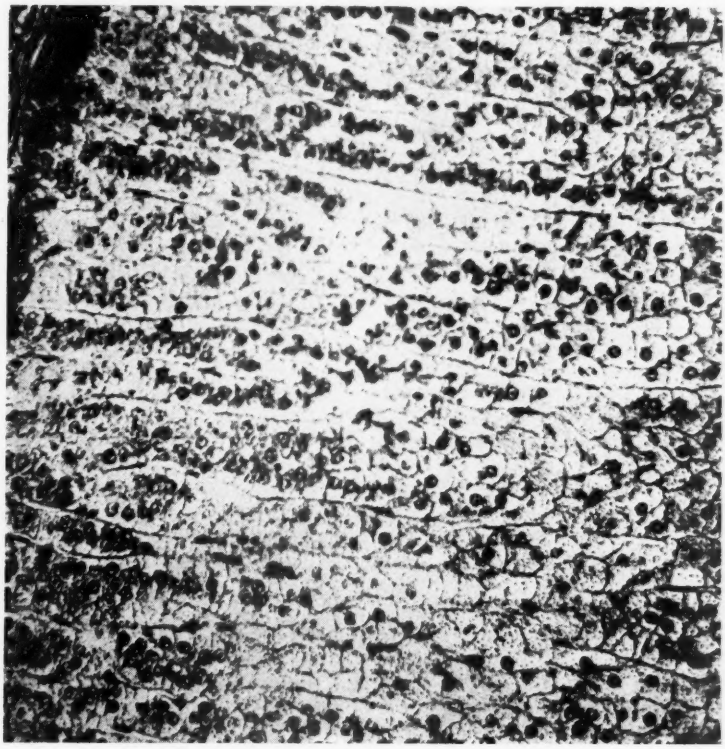

Fig. 2. — Photomicrograph showing a section of the suprarenal gland of rabbit No. 66 with severed left vagus-nerve. It received 400 units of insulin per kg one week after the operation and was killed 12 days after the injection. × 280.

SUMMARY

Tests were performed on ten male rabbits each weighing about 2 kg, one of which had the right and the rest of them the left vagus-nerve severed in the neck. The purpose of this investigation was to discover wether the operation on the vagus-nerve affected the ability of the animals to endure large doses of insulin.

It would appear that if the animals had fully recovered from the operation their ability to endure the insulin injections was similar to that of animals on which the operation had not been performed. (Noticeable individual differences could be observed). It would seem that the operation on the vagus-nerve did not pre-

vent the reaction in the organism aimed to check the action of insulin, which is shown by the changes found in the cells of the pituitary and the suprarenal glands after insulin injections.

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FROM THE STATE SERUM INSTITUTE, HELSINKI

SERUM CONCENTRATION OF DIFFERENT COMMERCIAL PENICILLIN PREPARATIONS

I

PROCAINE PENICILLIN G IN AQUEOUS OR OILY SUSPENSION

by

TAPIO SAVOLAINEN and VEIKKO TOMMILA

(Received for publication September 17, 1954)

Since the introduction of penicillin into general therapeutic use, its mechanism of action, dosages and modes of administration have been subjects of extensive study. As the administration of penicillin by injection at short intervals, usually at 3 hours, has proved inconvenient in many ways, attempts have been made to lengthen the intervals and yet maintain an adequate penicillin concentration in the organism throughout the period of treatment by, *e.g.*, the following means:

1) Retardation of the resorption of penicillin from the site of injection by impairment of the local blood circulation by various means (7),
2) Retardation of penicillin resorption by the use of various vehicles (7),
3) Retardation of penicillin resorption by the use of various poorly soluble penicillin salts (7),
4) Retardation of the renal excretion of penicillin by the administration of, for instance, »Benemid» (2, 3, 4, 7).

The study of means for attaining a prolonged penicillin concentration in the blood has led to the development of a large number of different commercial penicillin preparations with varying properties. For instance, the oily suspension of penicillin maintains

the penicillin level in the blood for a longer time than the aqueous suspension (7). This effect has been further prolonged by the addition of aluminium monostearate to the preparation (6, 9, 10). The size of particles is also significant, for small particles are able to keep up the penicillin concentration longer than large particles (11). The patient's general condition also plays an important part. Thus Boger *et al.* and Boger (1, 5) found a considerable difference in the retention of penicillin according to whether their subjects were afebrile and ambulatory or in bed with pyrexia and pneumonia, the latter patients retaining the drug for a longer time.

PRESENT INVESTIGATION

For injections at long intervals, procaine penicillin G administered in different vechicles either alone or with sodium or potassium penicillin G is almost exclusively used in Finland at the present time. Since many scores of these preparations have been marketed in Finland and some few tens are still being sold, and since the clinical observations of some physicians seemed to point to considerable differences in the therapeutic effect, it appeared desirable to investigate whether the commercial penicillin preparations available in Finland have an equal therapeutic value when the serum penicillin concentration attainable is used as criterion.

For this study, which was carried out in 1952—1954, material purchases were made from different pharmacies and pharmaceutical wholesalers, by the usual procurement procedure of practitioners, of all available preparations for injection treatment containing penicillin only (procaine penicillin G in aqueous or oily suspension) and designated as effective by the manufacturers, either direct or through their agents. We tested a total of 16 different commercial preparations of procaine penicillin G in aqueous or oily suspension, which comprised all the preparations obtained by us at the time.

CASE SERIES

The test subjects were adults hospitalised in the Helsinki University Hospital for Ear, Nose and Throat Diseases and in the First Central Military Hospital.[1] The age of the patients ranged

[1] We gratefully acknowledge the kindness of Professor Y. Meurman, M.D., Chief of the Helsinki University Hospital for Ear, Nose and Throat Diseases, and Major-Gen. P. Somer, M. D., Chief Medical Officer of the Finnish Army, in placing at our disposal a sufficiently large series of patients.

from 16 to 70 years and their body weights from 35 to 95 kg. Each test subject was given an intramuscular injection into the gluteal region of 600,000 units of the penicillin preparation under test. Blood samples were taken immediately before the injection and 1, 2, 3, 6, 12 and 24 hours later. Each preparation was tested on several persons (an average on five persons per preparation) and the average of the results was calculated and shown by graph. The determinations were carried out on a total of 80 test subjects.

According to some investigators the resorption of penicillin is retarded by the increasing age of the patients (8). We placed no particular stress on this, nor on the possible variations in the theoretic concentrations due to differences in body weight. However, possibly due to the small number of patients used for each tested product, no uniform findings were made in this respect in our tests as the results appeared to counterbalance one another.

TECHNIQUE

All the penicillin determinations were carried out by the cylinder plate method of the WHO (12), subject to a few minor deviations. A new basic curve was worked out every third day, since deep-frozen batches of *Sarcina lutea* were serviceable for three days only (and not two weeks, as stated in the instructions). Daily controls were made of the purity of the *Sarcina lutea* cultures by subcultures and staining, and of the number of viable bacteria in each deep-frozen batch by means of cultures of dilution series. WHO's working standard was received from the Food and Drug Administration, Washington, D.C., but since the quantity available was insufficient, we employed as standard a sodium penicillin. The latter was compared from time to time with the WHO standard, basic curves were drawn up for both at the same time, and the curves obtained for the individual patients with each tested product were reduced to the basic curve of the WHO standard on the basis of the differences obtained.

We determined chiefly the penicillin concentrations in the serum but also the penicillin unit content of some of the products was determined, using the same technique. A given amount of product was added to a known volume of distilled water, in which the used amount of penicillin was readily soluble. By sufficient

dilutions the area of the basic curve was reached and the determinations were made.

RESULTS

The results are most clearly seen from the graphs of the mean concentrations, in which the abscissa is the time from the injection and the ordinate the penicillin concentration in the serum. To render the results more comprehensible, the minimum therapeutic concentration is shown in the graphs at 0.05 units per millilitre of serum. The selection of this point is obviously somewhat arbitrary, since the effective therapeutic concentration varies according to the case and to the species and strain of the bacterium. In syphilis therapy, for instance, 0.03 u./ml is regarded as the minimum therapeutic concentration (14, 16).

Aqueous Suspensions with Procaine Penicillin G. — There were a total of 10 preparations which contained only procaine penicillin G in aqueous suspension. The results are shown in Figs. 1 and 2 and table 1.

In fig. 1 the results are given in the form of mean values, which can be evaluated better if the variations in the results obtained for the individual patients with the same product and the stantard

TABLE 1[1]

TESTED PROCAINE PENICILLIN PREPARATIONS IN AQUEOUS SUSPENSION, NUMBER OF PATIENTS, AND MEAN PENICILLIN CONCENTRATIONS IN SERUM AND THEIR STANTARD DEVIATIONS AT TIME OF MAXIMUM CONCENTRATION AND AT 24 HOURS

Preparation	No. of Patients	Mean Maximum Concentration		Mean Concentration at 24 Hours	
		u./ml. of Serum	Stantard Deviation	u./ml. of Serum	Stantard Deviation
C_2	5	0.96	±0.24	0.17	±0.06
U	5	0.49	±0.12	0.21	±0.03
G_1	6	0.41	±0.11	0.10	±0.03
C_1	5	0.30	±0.05	0.11	±0.02
I	5	0.30	±0.09	0.20	±0.04
E_1	5	0.23	±0.04	0.05	±0.02
F_1	5	0.19	±0.03	0.15	±0.01
A_1	3	0.20	±0.07	0.10	±0.01
D_1	8	0.20	±0.04	0.10	±0.02
B_1	2	0.14	±0.06	0.07	±0.03

[1] We gratefully acknowledge the assistance of Mr. E. Kaila, Ph.D., in controlling the mean values and calculating the stantard deviations.

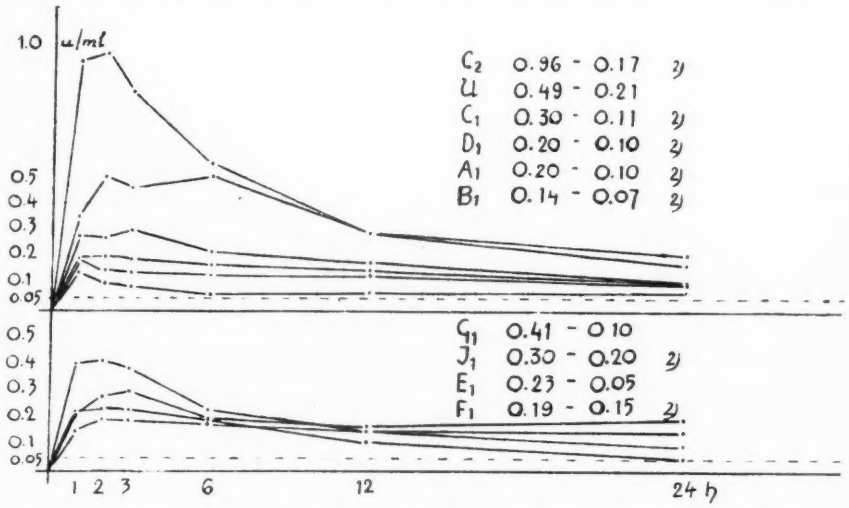

Fig. 1.[1] — Penicillin concentrations in sera of adults during 24 hours after a single intramuscular injection of 600,000 units of various commercial preparations containing only procaine penicillin G in aqueous suspension.

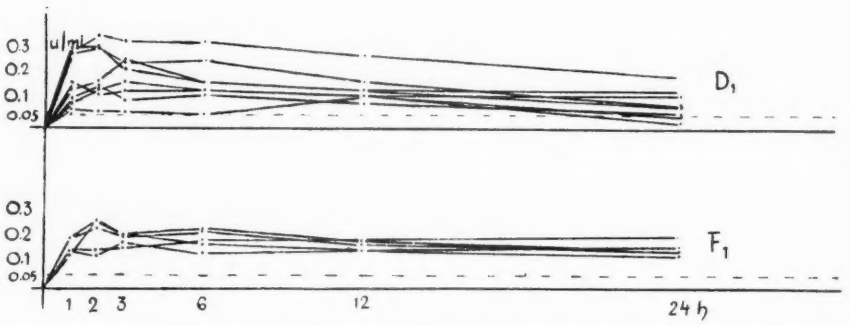

Fig. 2. — Individual penicillin concentrations in sera of adults during 24 hours after a single intramuscular injection of 600,000 units of preparations D_1 (8 patients) and F_1 (5 patients) containing procaine penicillin G in aqueous suspension.

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[1] The preparations are designated by letters; small figures indicate the numbers of the products of the same manufacturer. In the following columns are given the mean maximum concentration (in units per ml of serum) obtained with each preparation and the mean concentration (in units per ml of serum) 24 hours after the injection.

[2] Determinations made by the manufacturer after our tests were carried out gave for this batch of product B_1, according to the manufacturer's statement, a penicillin content of 350,000 u./ml, the declaration on the product being 300,000 u./ml. In our determinations the penicillin contents of preparations A_1, C_1, C_2, D_1, F_1, and I were 350,000, 317,000, 300,000—360,000, 300,000—310,000, 350,000—380,000, 300,000—360,000 u./ml, respectively.

deviations of the mean values are known. In fig. 2 are shown the results obtained for each patient with two products (D_1 and F_1). Of the 10 aqueous suspensions of procaine penicillin G tested, D_1 gave the greatest and F_1 the smallest variations between the individual patients. For the other products the differences lay within these limits. The stantard deviations of the mean values of the 10 products are shown in tables 1.

Oily Suspensions with Procaine Penicillin G. — There were altogether 6 preparations which contained only procaine penicillin G in oily suspension. The results are given in Figs. 3 and 4 and table 2.

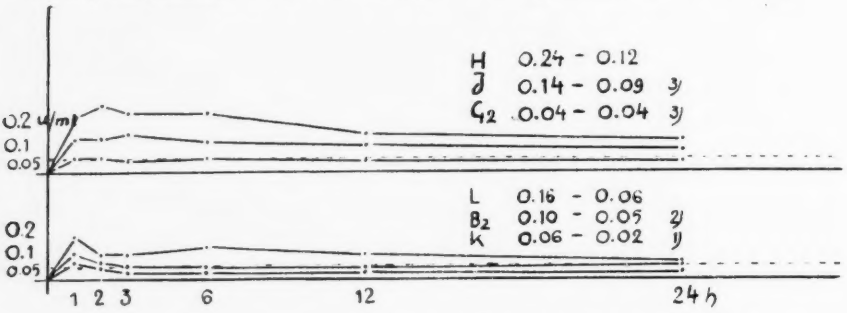

Fig. 3. — Penicillin concentrations in sera of adults during 24 hours after a single intramuscular injection of 600,000 units of 6 commercial preparations only containing penicillin G in oily suspension.

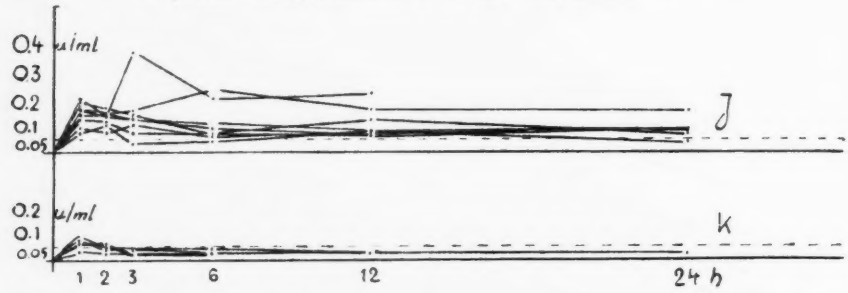

Fig. 4. — Individual penicillin concentrations in sera of adults during 24 hours after a single intramuscular injection of 600,000 units of preparations J (8 patients) and K (5 patients). Of all the tested preparations containing procaine penicillin G in oily suspension, J gave the greatest and K the smallest variations between the individual results of patients.

[1] According to the statement of the manufacturer, a unit content of 300,000—304,000 u./ml was obtained for this batch of preparation K.

[2] According to the statement of the manufacturer, determinations made at the factory after our investigation gave for preparation B_2 a content of 316,000 u./ml.

[3] In our determinations the penicillin contents of preparations G_2 and J were 340,000 and 300,000—340,000 u./ml, respectively.

TABLE 2

TESTED PROCAINE PENICILLIN PREPARATIONS IN OILY SUSPENSION, NUMBER OF PATIENTS, AND MEAN PENICILLIN CONCENTRATIONS IN SERUM AND THEIR STANTARD DEVIATIONS AT TIME OF MAXIMUM CONCENTRATION AND AT 24 HOURS

Preparation	No. of Patients	Mean Maximum Concentration		Mean Concentration at 24 Hours	
		u./ml. of Serum	Stantard Deviation	u./ml. of Serum	Stantard Deviation
H	3	0.24	±0.10	0.12	±0.01
L	3	0.16	±0.03	0.06	±0.02
J	8	0.14	±0.02	0.09	±0.01
B_2	6	0.10	±0.03	0.05	±0.01
K	5	0.06	±0.01	<0.02	—
G_2	6	0.04	±0.01	0.04	±0.01

DISCUSSION

The value of the investigation carried out by us lies essentially in the circumstance that comparisons were made of different commercial preparations of penicillin by the same investigators, by one technique, at one time, and with similar series of hospital patients.

From figs. 2 and 4 are seen the limits within which analogous results are obtained with the same preparation in different patients, while tables 1 and 2 show the stantard deviations of the mean values for each product at the time of maximum concentration and at 24 hours after injection.

All the preparations consisting of aqueous suspensions of procaine penicillin G administered in a single intramuscular injection of 600,000 units maintained during 24 hours a penicillin concentration in the serum which exceeded the minimum therapeutic concentration. In the case of some preparations the level at 24 hours was considerably over the minimum therapeutic level.

Compared with the results for aqueous suspensions, the oily suspensions of procaine penicillin G administered in the same manner gave in most cases definitely lower concentrations. It is especially noteworthy that three of the six preparations tested (B_2, G_2 and K) barely exceeded or attained the minimum therapeutic concentration. Because of the low concentrations these three preparations, at least from the batches examined by us,

cannot be recommended for the treatment of hospital patients. The remaining three preparations in this group (H, J and L) attain the level of the medium high preparations in the preceding group.

The maximum concentrations attained with the aqueous suspensions showed over 7-fold differences between the different preparations (B_1=0.13 u./ml as compared with C_2=0.96 u./ml). At 24 hours the differences also were 4-fold (E_1=0.05 u./ml as compared with U 0.21 ul./ml). The oily suspensions showed 6-fold differences at the maximum concentrations (G_2=0.04 u./ml as compared with H=0.24 u./ml), and over 6-fold differences at 24 hours (K= $<$0.02 u./ml as compared with H=0.12 u./ml). A comparison of all the preparations tested shows 24-fold differences in the maximum concentrations and over 10-fold differences in the 24 hour concentrations.

Nearly all the preparations reach the maximum concentration in the serum rapidly, *i.e.*, not later than within 3 hours after injection (7 preparations in 1 hour, 6 preparations in 2 hours and 3 preparations in 3 hours).

Earlier investigations also have revealed differences between various penicillin preparations (5). Reference may also be made to reports published by WHO (13, 15, 16) on tests with 9 oily suspension preparations of procaine penicillin (PAM penicillin) in a dosage of 300,000 units and using an observation time of 96 hours. At 24 hours the concentrations obtained with the different preparations varied between 0.02 and 0.22 u./ml of serum and at 96 hours between 0.00 and 0.08 u./ml of serum.

In view of the differences in the results the various penicillin preparations cannot be considered to be of equal therapeutic value. The variability in the results as well as other reasons point to the necessity for continued investigations of this kind.

SUMMARY

1. This report covers the results of tests made with 16 commercial penicillin preparations (10 preparations of procaine penicillin G in aqueous suspension and 6 preparations of procaine penicillin G in oily suspension) on 80 patients by the cylinder plate method. A single intramuscular injection of 600,000 units was given in the gluteal region and the penicillin concentration in the serum was

determined at various intervals during 24 hours. It was the purpose of the investigation to determine whether the various penicillin preparations have an equal therapeutic value when the serum penicillin concentration is used as criterion.

2. The results obtained show marked differences both between all the different preparations tested and between the individual preparations within each product group. Thus the choice of preparation in each case is not unimportant from a therapeutic point of view. The »best» results were obtained with preparations containing procaine penicillin G in aqueous suspension (giving a maximum concentration of 0.96 u./ml of serum and a concentration at 24 hours of 0.17 u./ml of serum). The procaine penicillin preparations in aqueous suspension were on the whole markedly superior to the oily suspensions. Three of the 6 preparations of the latter type gave, at least from the batches tested by us, such low penicillin concentrations in the serum that they cannot be recommended for hospital use.

3. All the tested preparations attained their maximum concentrations rapidly, usually within 1—3 hours.

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ESTIMATION OF THE VOLUMES OF THE CORTEX AND MEDULLA FROM ADRENAL SECTIONS

by

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A change in the adrenal weight may be due to a change in the weight of the cortex, in that of the medulla or in both. Since in most animals the medulla is small as compared to the cortex, the weight of the whole gland is in many instances a sufficiently accurate measure of the cortical weight. In the rat, e.g., the medullary volume is roughly one tenth of the cortical volume. A 50 per cent increase in the adrenal weight may then be due, e.g., to an increase by ca. 500 per cent of the medullary weight — which only occurs in very exceptional conditions, if ever — or to an increase in the cortical weight by some 50 per cent, an alternative mostly in question.

While frequently it thus is enough to estimate the size of the adrenal cortex by weighing the whole gland, an estimate of the medullary size cannot be obtained with the aid of a balance, wherefore its volume must be assessed by other means. The most accurate method of measuring the medullary volume is to measure the area of the medulla in a complete series of sections (6, 7). The sum of the medullary areas in all sections multiplied by the section thickness then gives the volume. This method is fairly tedious, which fact may explain the small proportion of recent papers dealing with the volume of the adrenal medulla, in comparison to those giving data of the weight of the whole gland.

Some less cumbersome methods for estimating the medullary volume have been used. One of the most popular ones is to measure

the size of the medulla in a »mid-section» only (e.g. 1, 3). This method is certainly convenient enough but its accuracy is open to doubt. Donaldson (2) has described a technique, in which the adrenal is made transparent by a clearing procedure, after which the outlines of the cortex and medulla are projected and drawn in two planes perpendicular to each other (see also 5). The volumes are then calculated from the areas measured. It is doubtful whether the real corticomedullary junction can be accurately seen in a cleared preparation. Moreover, even if that would be the case, the convex outer surface of the cortex acts as a lens distorting the outlines of the medulla.

Rather (8) has presented an interesting technique for estimating the corticomedullary ratio. Rat adrenals fixed in Bouin's fluid were cut into three or four discs, embedded in paraffin wax, and cut serially. The magnified images of the medulla and cortex were drawn on paper, and the respective areas were measured with a planimeter. The ratio cortical area/medullary area was determined on one slide, and the ratio plotted against the whole area. The procedure was then repeated with the next slide and the ratios were cumulated and plotted against the area. The lines connecting the cumulating ratios tend to become flat when a sample large enough has been taken, and the sampling is stopped when the cumulative ratio is regarded sufficiently stable. While the accuracy of this method may approach that of a complete measurement in serial sections, it seems open to doubt whether it will in practice be more convenient than the latter, which can be much simplified without noticeable loss of accuracy (see below).

In view of the considerable number of publications using the information obtained by measuring the »mid-section» and the convenience of this method, it was desired to study the adequacy of this kind of estimate in rat adrenals. Results obtained by comparing statistically the volume estimates calculated from mid-section areas with actual volumes measured from a complete series of sections are described in this paper.

MATERIAL AND METHODS

Altogether 60 adrenals from equally many animals were examined. 30 adrenals belonging to the first series were fixed in 10

per cent formalin for 24 hours and cut with a freezing microtome at 40 μ. The sections were projected through a low-power microscope on paper, and the outlines of the medulla were drawn in every section. The areas were then measured with a planimeter and the sum of areas in each gland, expressed in arbitrary units, was then used as a measure of the volume of the medulla. After the measurements this volume was plotted against the square root of the cube of the area of the section measured to have the largest medullary area in each gland. The regression was then analysed statistically.

Another series of 30 adrenals were fixed in a mixture of 1 part of concentrated formalin and 9 parts of 3.5 per cent potassium dichromate for 1 day and chromated in 3.5 per cent potassium dichromate for 3 days. This kind of treatment gives a suitable consistency to the glands so that they can be cut with a freezing microtome with little error in the section thickness. The fixed glands were weighed and sectioned serially at 50 μ. The sections were mounted unstained in glycerol. The magnified image of each section was then projected through the microscope on a horizontal glass tablet ground from the upper surface. The images were clearly visible from above and allowed direct planimetric measurements without drawing. The areas of the whole sections and those of the medullary parts were then measured by circumscribing each section in turn, always starting and finishing on the same point. The reading of the measuring wheel was read first after all sections in each gland were circumscribed. To get the volume in terms of mm^3 the sum of the measured areas (mm^2) was divided by the square of the linear magnification used (21.8 X) and multiplied by the section thickness (0.05 mm).

As the method of selecting the largest section was in the first series based on actual measurement, which will not be performed when the volume really is estimated only from one section, the largest section was chosen subjectively in the latter series. To get an idea of the additional variability thus introduced, three largest sections of each gland were selected and their whole and medullary areas measured. The first section thus selected of each gland is in the following text called »mid-section», whether from the middle of the gland or not.

In some glands, planimetric measurements were repeated once or twice. As the differences between the values obtained in measure-

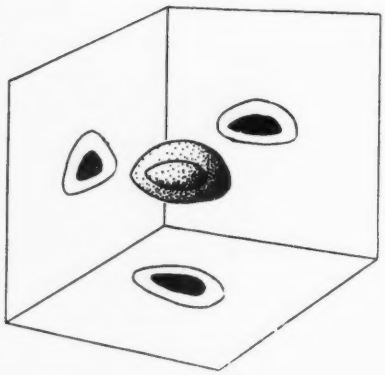

Fig. 1. — Projections of cortical and medullary areas of the rat adrenal on three perpendicular planes.

ments from the same gland always kept well below 0.5 per cent of the mean value, the rest of the glands were only once measured.

Some difficulties were met in the orientation of the adrenals for cutting. Fig. 1 illustrates the form of the rat adrenal. The mid-sections have different areas in different planes, and failure to recognize this fact results in a large variation entirely unrelated to the size of the whole gland or of the medulla. In the present study, painstaking efforts were made to cut the adrenals always in the same direction, i.e. the lower surface of the gland, which is against the kidney *in situ*, horizontal on the tissue holder of the microtome.

The volume estimates from the mid-sections were obtained, supposing the gland and the medulla to be rotation ellipsoids, by using the following equation:

$$V = \frac{4}{3\sqrt{\pi}} \sqrt{a^3},$$

where a is the measured area.

Regression equations and correlation coefficients were calculated supposing a linear dependence between the variates. The explanation capacities of the regression equations thus obtained were studied by the usual methods of the analysis of variance (4).

RESULTS

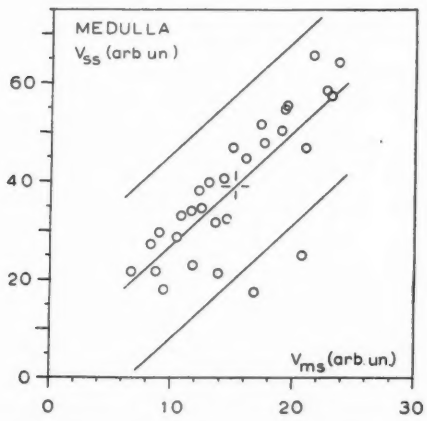

Fig. 2. — Regression of the medullary volume measured from serial sections (V_{ss}) on the volume estimate (V_{ms}) from the medullary area of the mid-section.

TABLE 1

REGRESSION OF MEDULLARY VOLUME ON VOLUME ESTIMATE FROM MID-SECTION IN SERIES I

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance	v^2	P
Regression	3 669.26	1	3 669.26	43.44	<0.001
Residual	2 364.95	28	84.46	.	.
Total	6 034.21	29	.	.	.

Correlation coefficient = +0.78.
Residual standard deviation, per cent of original s.d. = 64 %

1. *Size of Medulla.* — Figure 2 illustrates the dependence of the volume of the medulla, measured from a complete series of sections (V_{ss}), on the volume estimate obtained from the mid-section (V_{ms}), both expressed in arbitrary units, using different units for V_{ss} and for V_{ms}. The middle line shows the regression of the measured volume on that estimated from the middle section. The upper and lower lines, parallel with the regression line, indicate the fiducial limits at the 5 per cent level, i.e. their vertical distance from the regression line is obtained by multiplying the residual variation of V_{ss}, after fitting of the regression line, by the value of Student's t corresponding to $P = 0.05$ at 28 degrees of freedom. The width

of the belt bordered by these lines gives an idea of the goodness of the fit. Table 1 shows the corresponding analysis of variance.

It may be seen that, at the mean value of the volume estimate, V_{ms}, the real volume lies in 95 cases out of 100 between 20.1 and 57.5 units, while in 5 per cent of cases the true value will be farther away from the mean, 38.8 units. The fiducial limits are thus very large, a fact which can also be observed by comparing the magnitude of the residual standard deviation, 9.2, with the original standard deviation, 14.4. After fitting of the regression line most of the original variability in the volume remains unexplained, the regression explaining only some 35 per cent of the original standard deviation. Still, the regression is highly significant, the probability that the regression is due to mere chance being far less than 1 out of 1,000 ($P < 0.001$). The correlation coefficient is + 0.78 signifying a close interdependence of the variates.

Figure 3 and Table 2 illustrate similarly the regression in the other series, both the measured volume and the volume estimated from the mid-section being now expressed in mm^3, which allows direct comparison of the numerical values. Though the data are in this series obtained entirely independently of the first series and with slightly different methods, the results are closely similar: the

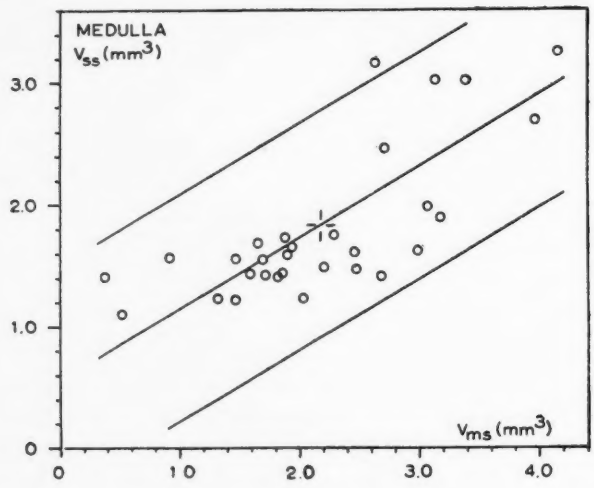

Fig. 3. — Similar regression as that shown in Fig. 2. from another set of 30 adrenals. In both cases the variations from the regression line are large.

TABLE 2

REGRESSION OF MEDULLARY VOLUME ON VOLUME ESTIMATE FROM MID-SECTION IN SERIES II

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance	v^2	P
Regression	8.1012	1	8.1012	39.52	<0.001
Residual	5.7390	28	0.2050	.	.
Total	13.8402	29	.	.	.

Correlation coefficient = 0.77.
Residual standard deviation, per cent of original s.d. = 65%.

correlation coefficients, the variance ratios (v^2), the relative fiducial limits and residual variations hardly differ from each other in the two series.

Though the regressions are in both series highly significant and a relatively high positive correlation exists between the variates, the above analysis shows clearly that the value of the medullary volume obtained from serial sections can only very roughly be estimated from the corresponding area in the mid-section.

2. *Size of Cortex and Whole Gland.* — In the second series of glands the accuracy of the estimate of the cortical volume was also studied. The results are given in Figure 4 and Table 3. If the estimate

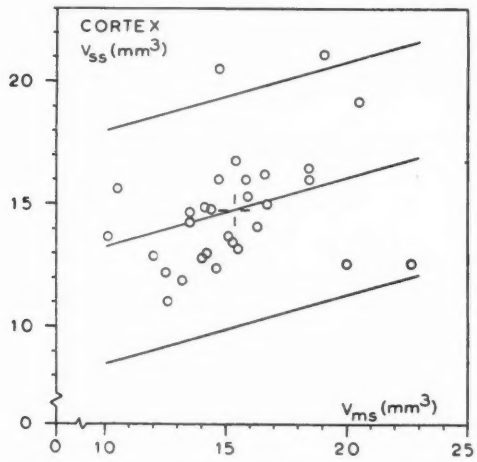

Fig. 4. — Regression of the cortical volume measured from serial sections (V_{ss}) on the volume estimate (V_{ms}) from the area of the mid-section.

TABLE 3

REGRESSION OF CORTICAL VOLUME OF VOLUME ESTIMATE FROM MID-SECTION

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance	v^2	P
Regression	18.43	1	18.43	3.43	>0.05
Residual	150.46	28	5.37	.	.
Total	168.89	29	.	.	.

Correlation coefficient = 0.33
Residual standard deviation, per cent of original s.d. = 96 %.

of the medullary volume from a mid-section was poor, the corresponding estimate of the cortical volume is entirely useless: the regression is capable of explaining only some 4 per cent of the original variation in the volume of the cortex, the 95 per cent fiducial limits nearly enclosing the whole variation range, and the correlation between the measured volume and the estimated volume being not even statistically significant.

As can be expected in view of the just-mentioned finding, the volume of the whole gland can neither be estimated with high accuracy from the area of the midsection. The results given in Figure 5, and Table 4 show closely the same characteristics as those given for the cortex.

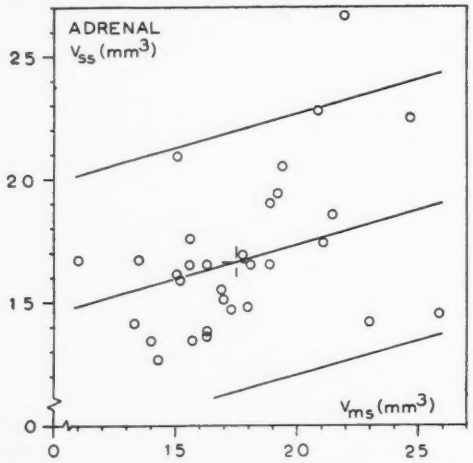

Fig. 5. — Regression of the adrenal volume measured from serial sections (V_{ss}) on the volume estimate (V_{ms}) obtained from the area of the mid-section.

TABLE 4

REGRESSION OF ADRENAL VOLUME ON VOLUME ESTIMATE FROM MID-SECTION
ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance	v^2	P
Regression	26.49	1	26.49	3.91	>0.05
Residual	189.84	28	6.78	.	.
Total	216.33	29	.	.	.

Correlation coefficient = 0.35.
Residual standard deviation, per cent of original s.d. = 95 %.

It may seem peculiar that the same method gives worse results when applied to the whole gland or to the cortex than when applied to the medulla. It must therefore be pointed out that the size of the medulla varied in the present series more than that of the whole gland or that of the cortex. While the largest value of the medullary volume was over 3 times as large as the smallest value, the ratios largest value/smallest value for the whole gland and for the cortex were smaller than 2. This difference at least partly explains the observed differences in the explanatory capacities of the regressions. In any case, the variability is too large to allow practical use of the mid-section estimate but under exceptional circumstances.

3. *Inaccuracy of Mid-Section Estimate.* — A poor correlation between the volume estimated from a mid-section and the volume measured with the aid of serial sections does not tell whether the former or the latter deviate more from the true volume. Consultation of data given in Figures 6 and 7 and in Tables 5 and 6 shows that there is no doubt about that the adrenal weight and thus, as there is no reason to suppose essential differences in the specific gravities of the glands, the adrenal volume are inaccurately estimated by the mid-section area.

On the other hand, the regression of the adrenal weight on the volume measured from serial sections is not only highly significant but shows only moderate residual variation after fitting of the regression line. Therefore, the volume obtained from serial sections can with some accuracy be used as a measure of the weight. The total residual variation in the regression of the weight on the measured volume is composed of two main components, of that due to error in the volume measurement and that caused by error in the

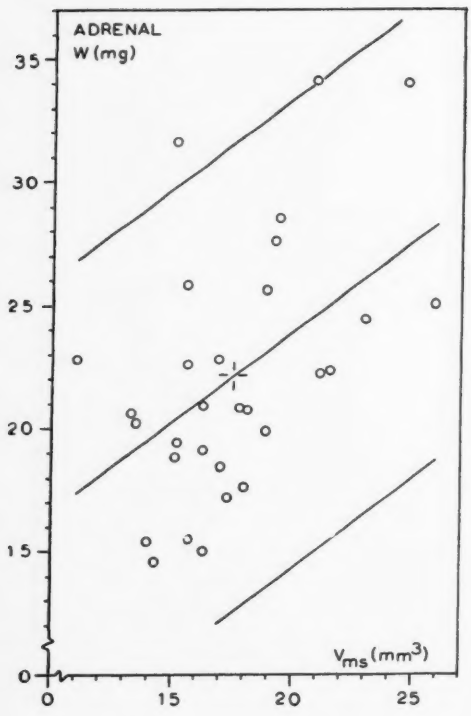

Fig. 6. — Regression of the adrenal weight (W) on the estimate of adrenal volume (V_{ms}) obtained from the area of the mid-section.

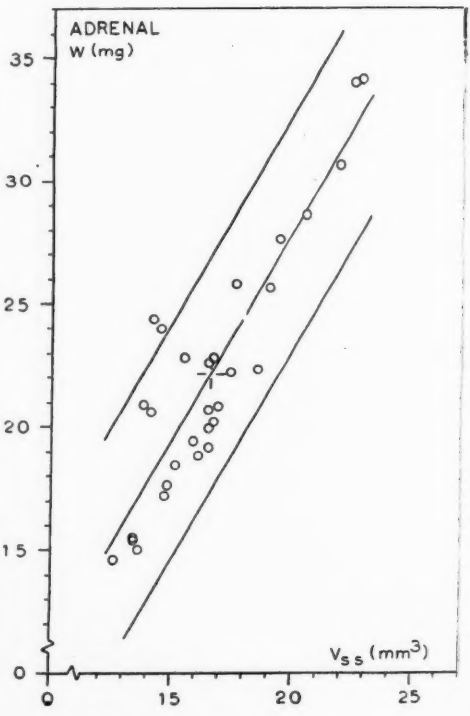

Fig. 7. — Regression of the adrenal weight (W) on the adrenal volume (V_{ss}) measured from serial sections.

TABLE 5

REGRESSION OF ADRENAL WEIGHT ON VOLUME ESTIMATE FROM MID-SECTION

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance	v^2	P
Regression	178.22	1	178.22	8.29	>0.001 <0.01
Residual	602.28	28	21.51	.	.
Total	780.50	29	.	.	.

Correlation coefficient = 0.48.
Residual standard deviation, per cent of original s.d. = 90 %.

TABLE 6

REGRESSION OF ADRENAL WEIGHT ON VOLUME MEASURED FROM SERIAL SECTIONS
ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance	v^2	P
Regression	624.49	1	624.49	112.12	<0.001
Residual	156.01	28	5.57	.	.
Total	780.50	29	.	.	.

Correlation coefficient = 0.89
Residual standard deviation ,per cent of original s.d. = 45%

weighing the gland. Consequently, the real error of the volume measurement is smaller than could be expected on the basis of the regression analysis. The accuracy of the measurement of the *medullary* volume, moreover, is very probably higher than that of the whole volume as some cortical sections were occasionally lost in the course of sectioning, which was in both ends rendered difficult, as the glands were not embedded. All sections from those parts of the gland containing the medulla were, on the other hand, safely included in the series. It may thus be concluded that the poor regression of the volume measured from serial sections on the mid-section area is essentially due to inadequacy of the latter criterion and not to inaccuracy of the volume measurement.

The low discrimination power of the mid-section may have been caused mainly by the irregularity in the form of the gland (see Figure 1) or by a failure to select a representative section. The results obtained in the first series of glands, in which the largest section was selected after actual measurements of the medullary areas, suggests that the former source of error is of major importance. This viewis further confirmed by the analysis of variance of the medullary and whole-section areas in triplicates, performed to get an impression of the magnitude of the error component introduced by the subjective selection of the largest section. Tables 7 and 8 show that this error can explain only a small fraction of the variability. Therefore, variations in the form, whose influence could not be prevented by trying to cut the glands always in the same direction, must be regarded as responsible for the failure of the mid-section estimate in guessing the adrenal or medullary volumes.

TABLE 7

ADRENAL AREAS IN MID-SECTION TRIPLICATES

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance
Between adrenals	472 592	29	16 365.2
Within adrenals, between sections ..	24 382	60	406.4
Total	498 974	89	.

Between-sections contribution to the standard deviation in a simple set of measurements $= \sqrt{\frac{406.4 \times 100.0}{16\,365.2 + 406.4}} = 16\,\%$

TABLE 8

MEDULLARY AREAS IN MID-SECTION TRIPLICATES

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Variance
Between adrenals	157 497	29	5 430.9
Within adrenals, between sections ..	6 135	60	102.3
Total	163 632	89	.

Between-sections contribution to the standard deviation in a simple set of measurements $= \sqrt{\frac{102.3 \times 100.0}{5\,430.9 + 102.3}} = 14\,\%$

DISCUSSION

The above results show that the mid-section areas of the whole adrenal, the cortex and the medulla are poor indices of the corresponding volumes. The accuracy of the volume estimate obtained from the mid-section is so low that statistically significant differences in the mid-section areas can only be expected when real differences in the volume are too large to be met but under extreme experimental conditions.

It is clear that some investigators are more skilled than the others. Therefore, it may well be possible that regressions better than those presented in this paper can be obtained by workers well-trained in the orientation of the adrenals for cutting. Moreover, the shape of the adrenal gland, which varies much from species to species and may be more ellipsoidical in some rat strains than in

others, is also of decisive importance in this respect. The above conclusions therefore apply only to animals with the same irregularities in the form of the adrenal than the rats used in this study. As the adrenal medulla of the mouse is very nearly ellipsoidical, at least in some strains, it is not surprising that Bullough (1) has apparently succeeded is estimating its size from the area of the largest section. On the other hand, it seems rather unlikely that the shape of the adrenal medulla of the rat will vary between different strains much enough to influence essentially the goodness of a volume estimate obtained from the mid-section area. This casts some doubt on the value of those earlier studies on the variations of the size of the rat adrenal medulla, in which the medullary volume was assessed from the mid-section.

In some circles it is believed that only paraffin sections can be used for accurate measurements of tissue components. This opinion may in some specific instances be right but it is certainly not generally applicable. It may be possible that the accuracy of the volume measurement had been somewhat increased in the present study by using paraffin instead of frozen sections. Nevertheless, no difficulties were encountered in the serial cutting at a constant speed of the fixed glands with the freezing microtome, and the percentage error in the thickness of the relatively thick sections may not be excessive in comparison to paraffin sections. A satisfactory regression of the adrenal weight on the volume measured from serial sections was indeed obtained, and errors in the section thickness could anyway not explain the distinct inferiority of the mid-section area as a weight indicator, in comparison to the complete series of sections.

Frozen sections, particularly thin sections from an incompletely fixed tissue, tend sometimes to enlarge in water after the cutting. The sections were in this study cut from properly fixed glands and they were relatively thick; accordingly, no variable spreading was seen after the cutting. Even if such a factor had been present it seems very unlikely that the degree of area change had varied much from one section to another, remembering that the differences in the triplicated mid-section measurements were relatively small.

It thus seems that the size of the adrenal medulla can be measured with sufficient accuracy only from serial sections, which inevitably means that one whole adrenal must be offered for this

purpose. The labour can nevertheless be reduced by using thick sections and by summing up the areas in each section mechanically with the aid of a planimeter, as described in this paper. When sections 50 μ thick are used, some 30—40 sections need to be circumscribed for measuring the medullary area in the rat, a labour not intractable. After the measurement, the same sections can be stained with various stains including those for the demonstration of lipids.

The volume measured from serial sections of fixed glands will of course not give the true volume of the gland during the life. Consultation of Figure 7 shows that a volume of ca. 17 mm^3 corresponds to a weight of ca. 22 mg. This would mean that the specific gravity of the gland would be 1.3 gm/cm^3, a value probably too high even considering the increase in the weight of the gland due to chromation. This seems to indicate that the fixation and chromation have caused some shrinkage. It is nevertheless reasonable to assume that the values obtained, though relative, are sufficiently informative for most purposes, large variations in the magnitude of the shrinkage seeming unlikely. Caution is necessary, however, in short-term experiments, in which the adrenal medulla is heavily stimulated. Such stimulation may cause a definite increase in the water content of the medulla, thus altering the readiness to shrinkage in various medulla, thus altering the readiness to shrinkage in various fixation fluids.

SUMMARY

The usefulness of the mid-section area as an indicator of the size of the adrenal medulla was studied in 60 serially cut adrenals.

A significant correlation was found between the estimate of medullary volume calculated from the mid-section area and the volume measured from a complete series of sections. However, in spite of the significant regression the residual variation left after fitting the regression equation was very large, indicating that the medullary area in a mid-section is a poor indicator of the medullary volume. Similar studies on the whole adrenal gland and the cortex gave similar results. Comparison of adrenal weights with the estimated or measured volumes showed moreover that area measurements in a complete series of sections give a reasonable estimate of the weight.

ACKNOWLEDGEMENTS

The writer is grateful to Mrs. Liisa Räisänen for careful technical assistance.

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FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF EDINBURGH, AND FROM THE PHYSIOLOGICAL DEPARTMENT, INSTITUTE OF OCCUPATIONAL HEALTH, HELSINKI

HISTOLOGICAL SAMPLING, CHROMATOGRAPHIC SEPARATION, AND DETERMINATION OF ADRENALINE AND NORADRENALINE IN THE ADRENAL MEDULLA

by

OLAVI ERÄNKÖ[1]

(Received for publication October 25, 1954)

In some previous studies the present writer (6, 7, 8) found two histochemically different types of cells in the adrenal medulla of the rat. After fixation in formalin, most cells showed but a faint fluorescence, but there were groups of intensely fluorescing cells forming clearly delineated islets. The assumption was made that this morphological dualism is related to the presence of two kinds of catechol amines in the adrenal medulla. Subsequent studies (10) have confirmed the presence of similar islets in other species whose adrenal medulla contains noradrenaline. It was therefore found advisable to study whether the distribution of noradrenaline is similar to that of the fluorescing islets. The results, which suggest the presence of high concentrations noradrenaline in the islet cells, will be reported elsewere.

Since the methods used for histological sampling and for the

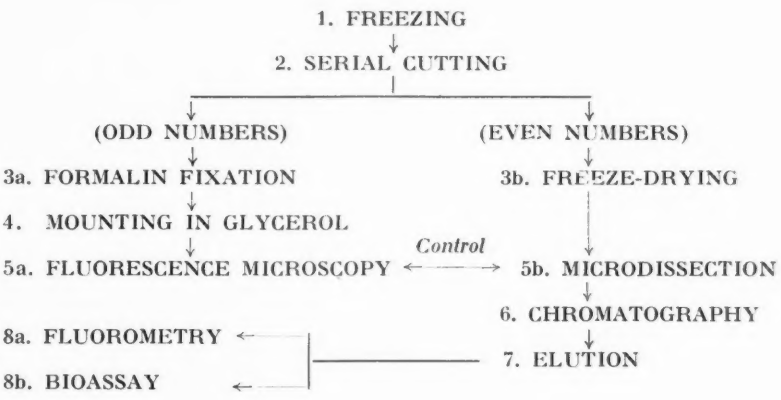
[1] Present address: Institute of Occupational Health, Työterveyslaitos, Helsinki. An essential part of this study was done during the academic year 1952—1953, which the writer spent as a British Council Scholar in the University of Edinburgh. The writer owes many thanks to Professor J. H. Gaddum, to Dr. Marthe Vogt and to Dr. T. B.B. Crawford for laboratory facilities and willing guidance. Mr. E. Siltanen and Mrs. L. Räisänen have given technical assistance, which is gratefully acknowledged. Financial support has been obtained from the Sigrid Jusélius Foundation and from the Valtion Luonnontieteellinen Toimikunta.

separation and determination of adrenaline and noradrenaline in the adrenal medulla may be of more general interest, they will be described in this communication. When the present work was in progress, Lowry (14) published an article on histological sampling for the study of the histochemistry of the brain. The sampling techniques used by Lowry and the present writer are in some respects similar, but the differences will be obvious from the following description.

EXPERIMENTAL

Comparison of the distribution of the fluorescing islets and that of the adrenal catechols is difficult because treatment with formalin, which is necessary for making the islets visible, destroys the catechols and because the islets cannot be demonstrated in sections from which the catechols have been extracted away. The only possibility is therefore to compare neighboring sections, one of which is treated with formalin for making the islets visible, the other one being studied for adrenaline and noradrenaline. The whole process is summarized in Table 1.

TABLE 1



Preparation of Freeze-Dried Sections. — Fresh adrenals were frozen on the tissue holder of an ordinary freezing microtome. Rapid freezing was conveniently done by applying a thin layer of distilled water on the tissue holder, by pushing the gland into

the water and by cooling the holder quickly with a liberal stream of carbon dioxide. Sections cut serially with a cooled knife were then one by one transferred with a brush into individual holes in a section holder kept at -78°C with the aid of dry ice. After completed cutting the section holder was transferred into a simple short-path freeze-drying apparatus (9), and the sections were dried at -40°C.

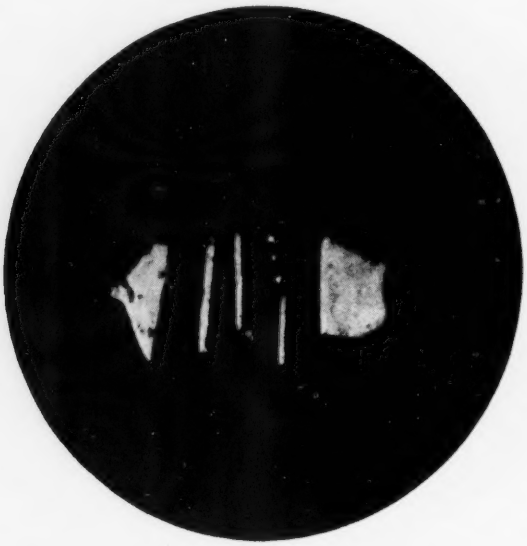


Fig. 1. — Low-power photomicrograph illustrating pieces of freeze-dried adrenal medulla cut free-hand with small scalpels prepared of a razor blade. 15 ×.

Dissection and Weighing. — The series of freeze-dried sections can of course be examined as such with chemical and histochemical methods. However, it was observed that the relative amounts of islet tissue and noradrenaline as a rule varied little from one whole section to another, thus allowing no conclusions about the relationships between the catechols and the islets. Therefore, dissection of small pieces from the adrenal medulla was indicated. The probable sites of fluorescing and non-fluorescing tissues in the freeze-dried section were determined by examining the two neighboring formalin-fixed sections for fluorescence. The corresponding parts in the freeze-dried section were then dissected out under a dissection microscope with the aid of a pair of small scalpels.

Preparation of pieces even less than 100 μ each way can be done manually without particular difficulties (Fig. 1). The pieces were subsequently weighed with a steel wire balance (20) or, if very small, with a quartz fiber balance (14).

Chromatographic Separation of Adrenaline and Noradrenaline. — The chromatographic method used was in principle similar to that developed for quantitative determinations in the Department of Pharmacology, University of Edinburgh, (2, 3, 17, 18, 21) from James's (13) qualitative method. In this method extraction and several purification stages precede the chromatography. As this preliminary treatment was tedious, it was examined whether it can be omitted in the study of adrenomedullary sections, containing high concentrations of catechols. To that end, freeze-dried pieces of the adrenal medulla were directly applied on the baseline of the paper previously moistened with a drop of 0.1 N hydrochloric acid. Whatman's No 1 paper washed overnight with 0.01 N hydrochloric acid was used. A cylinder was made by joining the lateral ends of the paper and the ascending chromatogram was developed at 26°C, using a mixture containing 85 per cent of phenol, freshly distilled in the presence of zinc powder, and 15 per cent (w/w) of 0.1 N hydrochloric acid. Chromatography was done under anoxic conditions by filling the tank with either nitrogen or carbon dioxide.

The paper cylinder was after development allowed to stay a few minutes on filter paper to drain off excess fluid. It was then cut to vertical strips, which were washed in several changes of analytical benzene until the smell of phenol vanished. One of the strips was then sprayed with a solution containing 0.44 per cent of potassium ferricyanide in a 0.4 M phosphate buffer and dried at about 100°C to develop fluorescence. The strip was examined in ultraviolet light, and the fluorescent spots were circumscribed with a pencil. When large quantities of catechols were present, the spots were visible in ordinary light but the fluorescence method is far more sensitive, making spots containing only some 0.1 μg of either catechol clearly visible. Narrow horizontal strips were then cut from the non-sprayed parts of the developed chromatogram, and these strips were eluted with a solution of 0.4 per cent sodium dihydrogen phosphate. The catechols were determined in the eluates either biologically or chemically (see below). Fig. 2

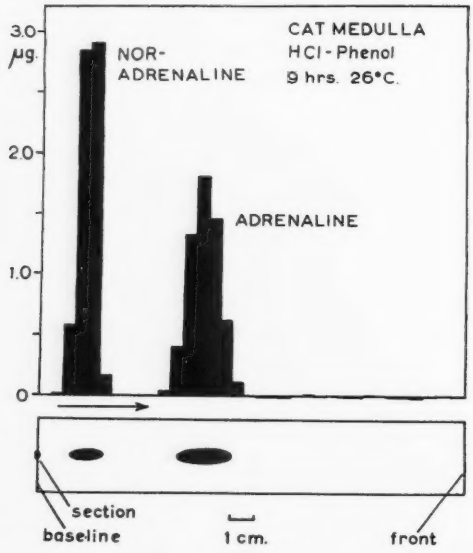


Fig. 2. — Above, distribution of adrenaline and noradrenaline in different parts of the chromatogram. Below, drawing prepared of the sprayed control strip showing the fluorescing spots as black. Note the localization of fluorogenic catechols exclusively in the regions characteristic to adrenaline and noradrenaline.

shows the result of a typical experiment. With sections 50 µ thick or thinner, practically all catechol activity was seen within a zone limited by lines some 0.5 cm from the extreme ends of the fluorescing spots in the sprayed strip. For routine estimations, 1.5 cm safety zones on both sides of the spots were used.

Both whole adrenal sections and their fragments can be treated in this way. As the presence of interfering substances in the section was doubted to influence the running speed, R_f values obtained with pure solutions of adrenaline and noradrenaline and with sections were compared. Lower R_f values were obtained with whole sections, while the running speed of the catechols from sections containing only the medullary part was the same as that of the catechols applied on the paper in pure solutions (Fig. 3). A third, clearly defined spot was always detected between the adrenaline and noradrenaline spots when cortical tissue was present in the section applied on paper; this spot was found to be due to ascorbic acid (Fig. 3), a fact of importance when fluorescence methods are

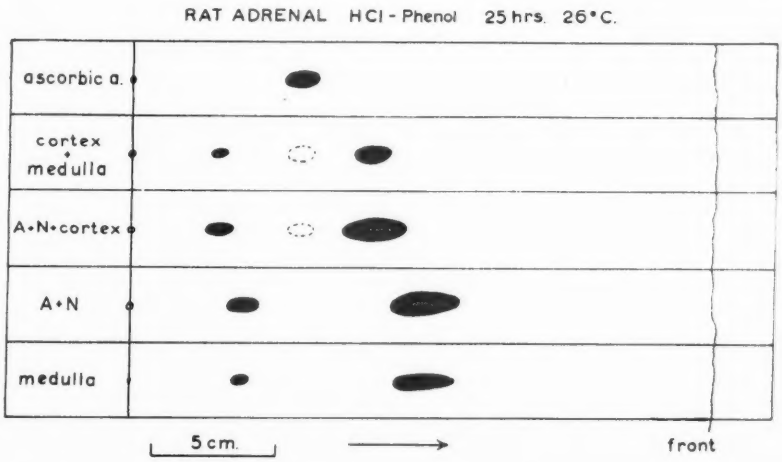


Fig. 3. — Negative tracing of a chromatogram. The uppermost strip was sprayed with dichlorophenol-indophenol and showed a white spot against blue background, illustrating the location of ascorbic acid. The other strips were sprayed with a buffered potassium ferricyanide solution. Adrenaline and noradrenaline were visible as strongly fluorescing spots, ascorbic acid as a white spot against yellow background. Note the retardation of adrenaline and noradrenaline spots and the presence of ascorbic acid in the strips with cortical tissue and the lack of such retardation in the strip with pure medullary tissue.

used for the determination of the catechols (see below). Pure ascorbic acid simultaneously applied with the pure catechols on the paper was also found to retard the running speed of the latter.

Biological Determination of Adrenaline and Noradrenaline. — The rat blood pressure assay (3, 17, 18, 21) was the only biological method used for the determination of noradrenaline, which can be estimated with this method in as small quantities as 0.005 μg. As about ten to twenty times as much adrenaline is necessary to obtain a reliable blood-pressure increase, the amount of adrenaline must be accordingly larger.

The rat was anaesthetized by injecting subcutaneously 0.7 cc of 25 per cent urethane (w/v) solution per 100 gm body weight. After the injection and during the assay the animal was kept warm with the aid of a tungsten lamp. The femoral vein was cannulated and the animal was heparinized and atropinized intravenously. The neck was then opened, both vagal nerves cut and the carotid artery cannulated. The blood pressure was registered with Condon's (1) manometer, giving nearly twice as big responses as an ordinary

U-shaped mercury manometer. To sensitize the blood pressure response, 1 or 2 mg of hexamethonium was injected intravenously (17). The first assay doses of catechols were injected some 15 min. thereafter, when the blood pressure had reached a steady level. Adrenaline or noradrenaline were always injected at constant intervals in a total volume of 0.3 ml, including the volume used for washing the plastic cannula. Known and unknown doses were injected alternatively. The assay was continued until the magnitude of the blood pressure response to the unknown dose was at least twice between the responses obtained with two known doses sufficiently near to each other.

When the eluate contained less than ca. 0.1 μg of adrenaline, rat's uterus assay was used. The assay (4, 5) is based on inhibition by adrenaline of uterine contractions induced by carbaminoyl choline. The sensitivity varies from one uterus preparation to another but 0.0001 μg can be assayed under favourable circumstances.

The biological methods are tedious and only few samples can be assayed on each preparation with sufficient accuracy. Whenever the amounts of catechols were large enough they were therefore determined chemically by the fluorescence method described below. Biological and chemical methods were found to give comparable results, the latter being nevertheless more accurate.

Chemical Determination of Adrenaline and Noradrenaline. — The method was based on the fluorescence method described by Lund (15, 16). This method makes use of the formation of a fluorescing compound, adrenolutine, from adrenochrome, which is an oxydation product of adrenaline. Lund (16) suggested differential determination of adrenaline and noradrenaline by oxidizing one half of the sample at pH 6 and the other half at pH 4 (see 12). Practically only adrenaline is oxidized at pH 4, while both adrenochrome and noradrenochrome are formed at pH 6. This kind of differentiation is too inaccurate in our experience (see also 19), particularly when the main object of the study is to register differences in the adrenaline/noradrenaline ratio. The determinations were therefore always done first after chromatographic separation of the catechols.

In earlier experiments, the eluates were oxidized according to Lund by shaking specially purified manganese dioxide with the

catechol solution for 30 sec. at pH 6, after which manganese dioxide was centrifuged off. Ascorbic acid was added to the clear supernatant to prevent further oxydation. The pH was then made strongly alkaline with sodium hydroxide and the fluorescence was measured with the Farrand fluorometer or with the Beckman photometer. For various reasons this method of oxydation was not entirely satisfactory. Manganese dioxide was therefore replaced with potassium ferricyanide, briefly mentioned by Euler (11) to give good results. The technique used in our laboratory at present is given below.

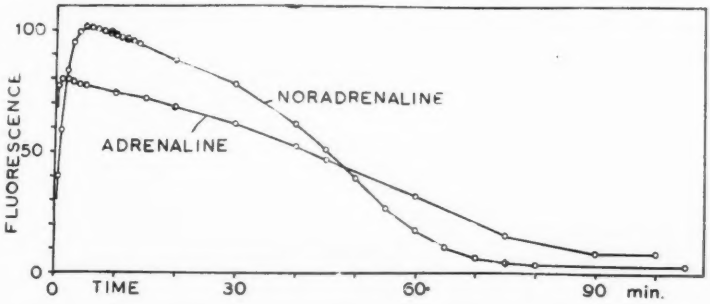


Fig. 4. — Intensity of fluorescence as a function of time after addition of sodium hydroxide in oxidized solutions of adrenaline and noradrenaline. The fluorescence intensity is expressed as per cent of that of the quinidine standard. It is seen that adrenochrome is quickly transformed to adrenolutine, while the maximum fluorescence with the noradrenaline solution is reached first some 5 min. after the addition of sodium hydroxide.

Elute the paper with 0.4 per cent sodium dihydrogen phosphate. Adjust the volume with the same solution to 3 ml. Add 0.75 ml of a 0.98 per cent solution of disodium hydrogen phosphate, mix and add 0.05 ml of 0.5 per cent potassium ferricyanide and mix immediately. After exactly one minute add 0.05 ml of 0.5 per cent ascorbic acid and mix immediately. Add 0.6 cc of 25 per cent sodium hydroxide, mix and measure the fluorescence against a water blank and a quinidine standard. The fluorescence intensity reaches its maximum soon after addition of sodium hydroxide (Fig. 4). The maximum fluorescence intensity is linear to the catechol concentration and the accuracy is satisfactory even with amounts of ca. 0.2 μg when the fluorescence outfit of the Beckman photometer is used. Smaller amounts can be measured with the Farrand fluorometer. Studies on chromatographed extracts of the

adrenal medulla failed to demonstrate the presence of any other catechols or interfering substances.[2] With extracts of the whole gland care must be taken not to elute the ascorbic acid off the paper, as this substance prevents the oxydation of adrenaline and noradrenaline.

Calibration lines were prepared in each set of determinations for both adrenaline and noradrenaline by measuring the intensities of fluorescence produced by 0, 0.5 and 2 μg of the catechols, respectively. The over-all standard error of the method, including errors in the pipetting of the mentioned amounts and those due to (negligible) deviation from the linearity amounted to $\pm$ 3.5 per cent for adrenaline and $\pm$ 2.8 per cent for noradrenaline, as calculated from 24 double determinations.

SUMMARY

A procedure is described for studying the quantitative distribution of adrenaline and noradrenaline in the adrenal medulla and for correlating the results with histochemical findings. Fresh organs are serially cut with a freezing microtome. Every other of the sections are examined histochemically after formalin fixation, the remaining sections being freeze-dried. From the dry sections are dissected small regions for chemical analysis. Adrenaline and noradrenaline are chromatographically separated from previously weighed small sections, which are as such applied on the base-line of the chromatography paper. Appropriate areas of the chromatogram are eluted and the amounts of adrenaline or noradrenaline are estimated either biologically or chemically. A sensitive fluorometric method suitable for this purpose is described.

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[2]) Mary Lockett (J. Physiol. 1954:*124:*67P) has recently reported the presence of a catechol in extracts of cat adrenals giving a fluorescent spot in the chromatogram at R_f 0.7 after spraying with potassium ferricyanide. The present writer, using identical techniques, has not been able to confirm this observation with sections of cat adrenal medulla.

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FROM THE DEPARTMENT OF PHYSIOLOGY, INSTITUTE OF OCCUPATIONAL HEALTH, HELSINKI

A NEW VENTILATORY DYSPNEIC INDEX[1]

by

TEUVO K. I. LARMI

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Studies on the mechanics of breathing revealed that each alveolar ventilation has a respiratory rate optimum when the muscular work used for breathing is smallest (1, 11). It was pointed out, further, that in various heart and pulmonary diseases the abnormal increase in the amount of work caused by breathing is an important factor causing dyspnea (3). Therefore patients suffering from pulmonary insufficiency attempt to avoid the greatest resistance from the point of view of respiratory work by adjusting their respiratory rate to it. The total amount of work caused by breathing is the sum of resistances due to elasticity of the chest wall and the lungs, to viscance and turbulence of the respiratory air, and to the inelastic changes of the tissues (11, 15). A certain relation of elastic restistance to the respiratory and lung volumes has also been observed (8, 13, 14, 15, 16). It seems likely, therefore, that the ratio of tidal air to vital capacity, expressed as a percentage of vital capacity, indicates a relative measure of work done in each single breath. The amount of this relative work per unit time, *i.e.*, the power of the relative work, is indicated by the expression

$$RR \times \frac{TA}{VC} \times 100 = \text{power},$$

[1] According to the paper read at the 7th Northern Meeting of the Laboratory Physicians, Upsala, Sept. 10—11, 1954.

in which RR denotes the respiratory rate, TA tidal air, and VC vital capacity. TA can be substituted by the expression $\frac{MVR}{RR}$, MVR being the minute volume of respiration. Thus, a plain expression for the power of work caused by breathing is obtained as follows: $\frac{MVR}{VC} \times 100 = \text{power}$.

This expression, simplified without a percentage coefficient, has been used as dyspneic index in the present study. Comparisons have been made between this and the index presented by Cournand and Richards (4), $\frac{BR}{MBC} \times 100$, in which BR denotes the breathing reserve and MBC the maximum breathing capacity.

MATERIAL AND METHODS

The investigations were performed on 92 subjects, divided into six groups on the basis of the following clinical criteria: 1) control group or the subjects without any pulmonary or heart diseases, 2) subjects with pulmonary disease without emphysema, 3) subjects with emphysema without heart symptoms, 4) subjects with cor pulmonale, 5) subjects with left heart failure without pulmonary disease, and 6) subjects with pulmonary disease and complicating left heart failure. In all the cases the *vital capacity* (VC) (lying) and *maximum breathing capacity* (MBC) (standing) were examined by means of a spirometer (Benedict Roth »Respirometer», Warren E. Collins, Boston, Mass., USA) and the values were corrected according to BTPS. The *minute volume of respiration* (MVR) was determined by a gasometer (10), the values being likewise corrected according to BTPS. The bicycle ergometer (5) and graduated exercise were used in examining the minute volume of respiration. Special attention was paid to the following points: 1) the heaviest exercise on which the subject did not show dyspneic symptoms (*the lower dyspnea threshold*), 2) the lightest exercise on which the subject showed dyspneic signs (*the upper dyspnea threshold*), and 3) The *duration of dyspnea* after the exercise had exceeded the upper threshold. Variance and covariance analysis were used as statistical methods. The material and the methods used have been reported in more detail in another connexion (7).

RESULTS

The results obtained represent the values of the summation of the upper and lower dyspnea thresholds. It was assumed that this summation, divided by two, indicates the index value in which

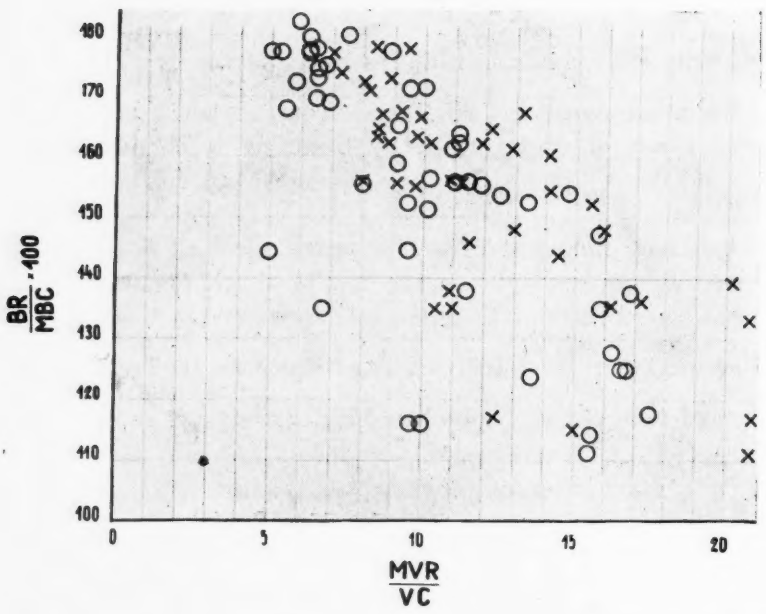

Fig. 1. — Correlation between the dyspneic indices $\frac{BR}{MBC} \times 100$ and $\frac{MVR}{VC}$ (as a summation of the upper and lower dyspnea thresholds), when the material was divided on the basis of the load producing dyspnea. ○ = Dyspnea appeared after the load of 300 kgm/min. × = Dyspnea appeared with the loads of 500—900 kgm/min. The regressions did not differ from each other.

dyspnea actually appears. However, the results corresponded entirely to the findings reported above, whether the values were obtained separately either from the upper or the lower threshold.

Fifty out of 92 subjects showed dyspnea with a load of 300 kgm/min., the others, 42 subjects, with a load of 500 kgm/min. or on heavier exercise. In both groups the correlation between $\frac{MVR}{VC}$ and $\frac{BR}{MBC} \times 100$ deviated highly significantly from zero, the correlation coefficients being $r = 0.621$ and $r = 0.665$, but covariance analysis revealed no significant difference between the regressions in the groups (Fig. 1).

The duration of dyspnea was less than one minute in 52 subjects, from one to two min. in 24 subjects, and more than two min. in 16 subjects. The correlation coefficients ($r = 0.415$, $r =$

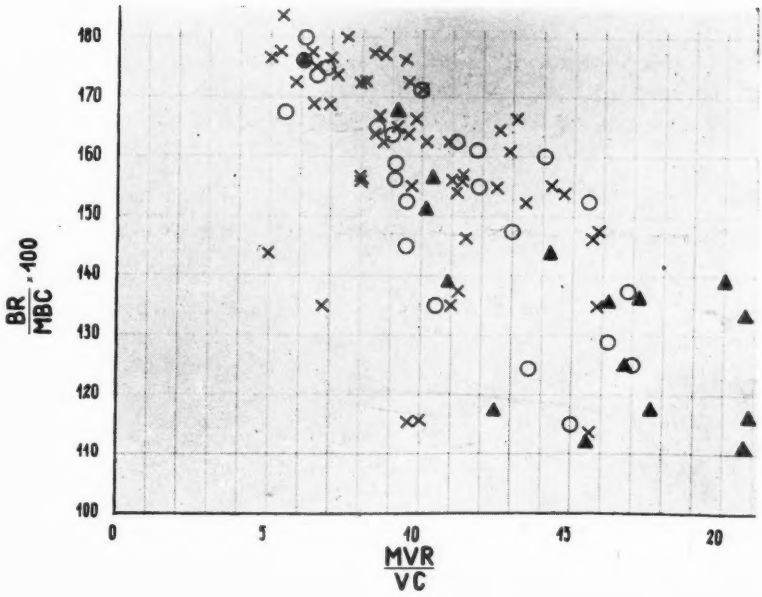

Fig. 2. — Correlation between dyspneic indices $\frac{BR}{MBC} \times 100$ and $\frac{MVR}{VC}$ (as a summation of the upper and lower dyspnea thresholds), when the material was divided according to the duration of dyspnea after a two-minute exercise. × = The duration of dyspnea was less than two minutes. ○ = The duration ranged from one to two minutes. ▲ = Dyspnea lasted longer than two minutes. The regressions did not differ from each other.

0.726, and r = 0.740, respectively) between the dyspneic indices deviated significantly from zero (p = 0.5 per cent, p = 0.4 per cent, and p = 1.9 per cent, respectively). The regression study showed, however, that directions of the regressions did not deviate from each other significantly (Fig. 2).

When the series was divided into six groups on the original clinical basis, it was observed that the correlation coefficient (r = 0.570) between the dyspnea indices $\frac{MVR}{VC}$ and $\frac{MBC}{BR} \times 100$ significantly deviated from zero in the entire series. Covariance analysis showed, however, that the regression between the means of the groups deviated highly significantly from that within the groups (Fig. 3). The finding demonstrated that the $\frac{BR}{MBC} \times 100$ index changed more when moving from one group to another than

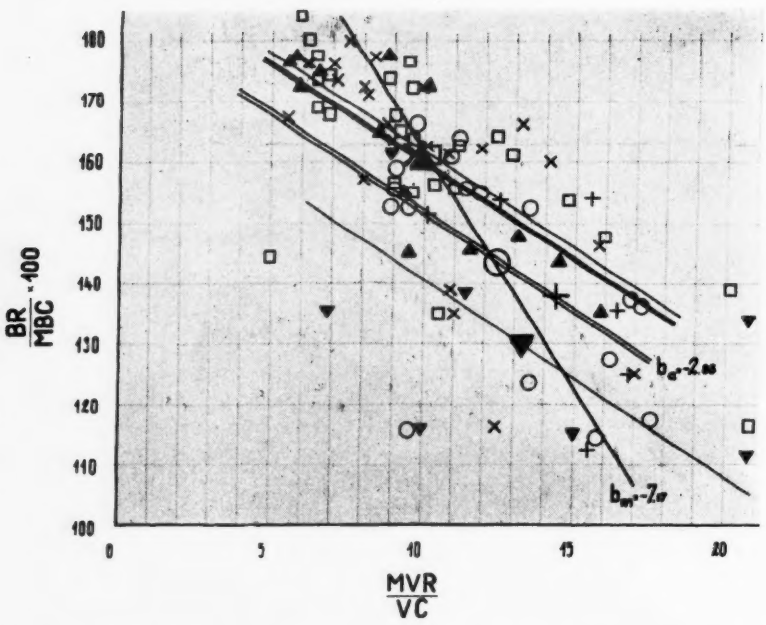

Fig. 3. — Correlation between the dyspneic indices $\frac{BR}{MBC} \times 100$ and $\frac{MVR}{VC}$ (as a summation of the upper and lower dyspnea thresholds), when the material was divided on the clinical basis. × = control group, □ = subjects with pulmonary disease without emphysema, ○ = emphysema group, ▼ = cor pulmonale group, ▲ = subjects with left heart failure, and + = subjects with pulmonary disease and complicating left heart failure. The large symbols indicate the means of the various groups, the small ones show the single observed values. The average regression within the groups (regression coefficient $b_a = -2.86$) and the regression between the means ($b_m = -7.17$) differed from each other highly significantly.

it did among the single subjects within each group. On the contrary, the $\frac{MVR}{VC}$ index changed more among the single subjects within the group than it did at the transition from one group to another.

Variance analysis was employed in the study of the *significance*, through which the new $\frac{MVR}{VC}$ and the old $\frac{MBC}{BR} \times 100$ indices separated the different divisions of the material from each other. Table 1 shows the average values of the indices and the significance of the differences regarding both the exercise producing dyspnea

TABLE 1

TABLE 1 INDICATES THE SIGNIFICANCE THROUGH WHICH THE DYSPNEIC INDICES $\frac{MVR}{VC}$ AND $\frac{BR}{MBC} \times 100$ (AS A SUMMATION OF THE UPPER AND LOWER DYSPNEA THRESHOLDS) SEPARATED THE GROUPS, WHEN THE SERIES WAS DIVIDED BOTH ON THE BASIS OF THE EXERCISE PRODUCING DYSPNEA AND THE DURATION OF DYSPNEA AFTER A TWO-MINUTE EXERCISE

Dyspneic Index	Dyspnea Appears at Load (kgm/min)		Difference		Duration of Dyspnea			Difference	
	300	500—900	v^2	p %	< 1 min	1—2 min	> 2 min	v^2	p %
$\frac{MVR}{VC}$	10.20 ± 0.56	11.85 ± 0.57	4.28	4.6	9.78 ± 0.41	10.95 ± 0.73	14.93 ± 1.17	13.67	<0.1
$\frac{BR}{MBC} \times 100$	153.6 ± 3.37	153.6 ± 2.77	no difference		158.9 ± 2.87	154.0 ± 3.70	136.4 ± 4.92	7.74	<0.1

and the duration of dyspnea after exercise. It was noted that the difference obtained by $\frac{MVR}{VC}$ was almost significant ($v^2 = 4.28$; $p = 4.6$ per cent) between the subjects who became dyspneic

TABLE 2

TABLE 2 INDICATES THE SIGNIFICANCE THROUGH WHICH THE DYSPNEIC INDICES $\frac{MVR}{VC}$ AND $\frac{BR}{MBC} \times 100$ (AS A SUMMATION OF THE UPPER AND LOWER DYSPNEA THRESHOLDS) SEPARATED THE CLINICAL GROUPS FROM EACH OTHER

Clinical Group	Number of Subjects	Dyspneic Index	
		$\frac{MVR}{VC}$	$\frac{BR}{MBC} \times 100$
I	22	9.75 ± 0.67	161.2 ± 3.08
II	27	10.22 ± 0.78	161.4 ± 2.91
III	17	12.38 ± 0.79	143.0 ± 5.55
IV	6	14.43 ± 1.11	138.5 ± 7.02
V	13	9.88 ± 1.03	160.7 ± 4.34
VI	7	13.40 ± 2.13	125.7 ± 9.55
Difference		3.17	7.63
v^2		1.0%	0.1%
p			

after the load of 300 kgm/min. and the subjects who did not show dyspneic symptoms until after heavier loads. The old $\frac{BR}{MBC} \times 100$ index did not reveal significant differences. Similar findings were also observed as to the duration of dyspnea. Both indices showed hihly significant differences; that obtained by $\frac{MVR}{VC}$ being stronger ($v^2 = 13.67$) than that obtained by $\frac{BR}{MBC} \times 100$ ($v^2 = 7.74$).

When studying the significance of the differences on the basis of the clinical division, it was noted that the differences obtained by the new index were almost significant ($v^2 = 3.17$; $p = 1.0$ per cent) between the various groups, while those obtained by the old index were highly significant ($v^2 = 7.63$; $p < 0.1$ per cent).

DISCUSSION

As a dyspneic index the ratio $\frac{MVR}{VC}$ is simpler than the ratio $\frac{MBC}{BR} \times 100$, and it is obvious that the former is a better indicator of dyspneic symptoms. The new index, however, has its drawbacks, as it is assumed in its derivation that inspiration alone demands active muscular work. In obstructive insufficiency and similar conditions, expiration, too, requires active work (2, 9, 12, 15). If dyspnea appears when the effectiveness of work used for breathing exceeds a certain limit, attention should also be paid to the role played by this work. It is possible that dyspnea, which is not pathological (6), can be explained in this way in further studies.

SUMMARY

1) The ratio $\frac{MVR}{VC}$, indicating the power of a certain relative work, was derived on the basis of work used for breathing due to elastic resistance of the lungs.

2) When $\frac{MVR}{VC}$ was compared with the former ratio $\frac{MBC}{BR} \times 100$, it was found that the regressions between them were divergent,

the series being divided on the clinical basis. No differences occurred, on the other hand, when the duration of dyspnea or the exercise producing dyspnea were the dividing criteria used.

3) The ratio $\frac{MVR}{VC}$ yielded more significant differences in the various groups than the ratio $\frac{BR}{MBC} \times 100$, when the series was divided on the basis of the duration of dyspnea or of the exercise producing it. On the other hand, MVR revealed less significant differences when the clinical basis was used as a dividing criterion.

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DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

EFFECT OF PSYCHIC STRESS ON THE CELLS OF THE PITUITARY BODY AND THE SUPRARENAL GLANDS OF RATS

by

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Although practising physicians have observed examples of the part played by psychic factors in the inception of disease ever since the early history of medicine, not until recently have efforts been made to probe deeply into their significance.

Numerous studies have been published in the last few years based on observations made in connection with autopsies. The endocrine glands of persons suffering from serious illness or other stress have been examined and lesions caused by stress noted.

Inasmuch as the conditions involved in the study of human beings are extremely complicated, it is wise to check results with experiments on animals. Experiments designed to ascertain the effect of psychic stress on animals have, however, seldom been performed. J. Schunk and H. Cornelius have studied the changes that take place in the pituitary body and suprarenal glands of cats upon their being confronted with their hereditary enemy, a dog. They arranged for four cats to spend two hours a day for a period of three months in the near presence of dogs. Marked changes could be noted in the pituitary bodies of the animals under observation. Particulary in the central part of the anterior lobe, a striking increase in the number of beta cells had taken place. Furthermore, degenerative changes could be perceived, notably in the beta and gamma cells. A shrinkage of the nucleus in the beta

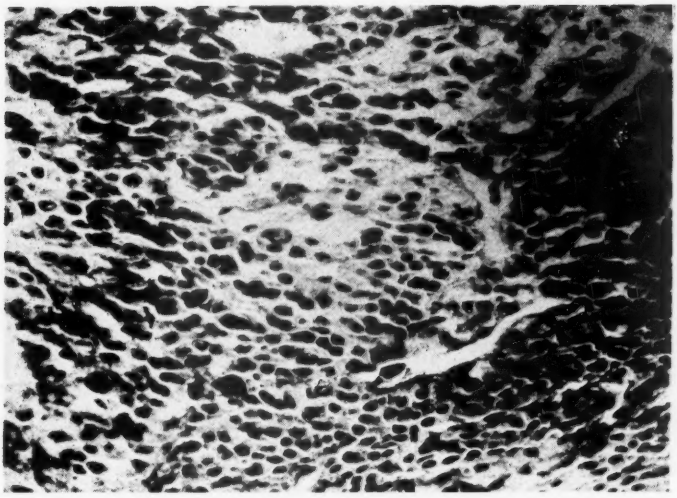

Fig. 1. — Photomicrograph showing a section of the central part of the anterior lobe of the pituitary body of a rat exposed to stress for a period of 30 days. The number of beta cells extraordinarily multiplied. Varying in size, these cells reveal different degenerative changes, atypic nuclei, vacuoles, etc. Many cells destroyed. ×500.

cells had occurred; the granules were in many cases missing, and the cells tended to vary in shape and size. Degenerative changes, notably vacuolization were observed also in the gamma cells. The changes in the alpha cells were slighter; the granules tended to be missing or atypic nuclei could sometimes be detected.

In the cortex of the suprarenal body of the test animals, a considerable loss of lipoid substances was noted, especially in the zona fasciculata. In the medulla of the suprarenal body, there could be observed a dilatation of the lumina in the veins and vacuolization of the cytoplasm in the cells of the medulla.

No hemorrhages or other signs of degeneration could be detected by these researchers in either the medulla or cortex of the suprarenal body.

MATERIAL AND METHODS

The series comprised 20 white male rats, averaging 200 g in weight. Various means were used to produce stress, as, for instance, brilliant flashes of light and loud modern dance music reproduced by recording discs. It was attempted to frighten some

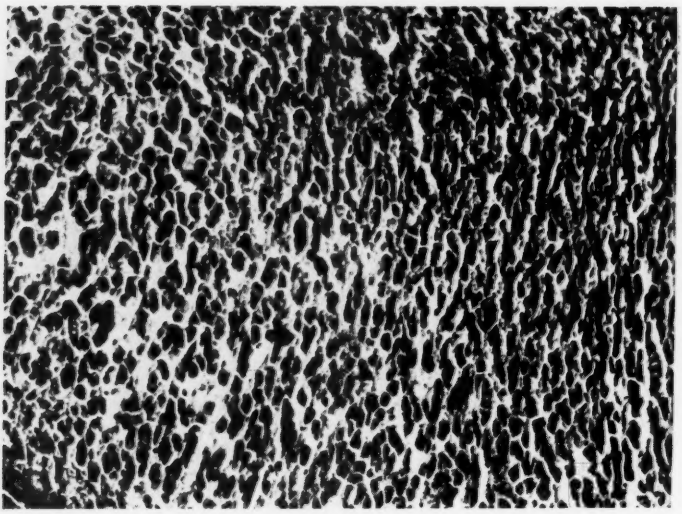

Fig. 2. — Photomicrograph showing a section of the suprarenal gland of a rat exposed to stress for 23 days. Necrotic cells and haemorrhages throughout the cortex. ×200.

of the rats by letting a cat walk around their cage; they were threatened with pincers. The rats proved remarkably well suited for experiments of this kind. They became readily irritated, rose up on their hind legs and often fought in pairs long after the external irritant had ceased to be applied.

The conditions of stress were maintained for periods ranging from 12 to 105 days, after which the rats were decapitated and their pituitary bodies and suprarenal glands promptly weighed. Afterwards the specimens were fixed in Zenker's fluid and embedded in the usual way. Specimens of the pituitary body were stained according to Mann's long method, whereas in staining the suprarenal glands primarily Delafield's haematoxylin-eosin was used.

RESULTS

PITUITARY BODY. — The weight of the pituitary body of the rats subjected to psychic stress ranged from 5.6 to 4.0 mg (compared to 4.4—2.7 mg for the control animals). In order to eliminate the effect on the results of differences in individual size, the weight of the pituitary body was calculated in ratio to the animal's total

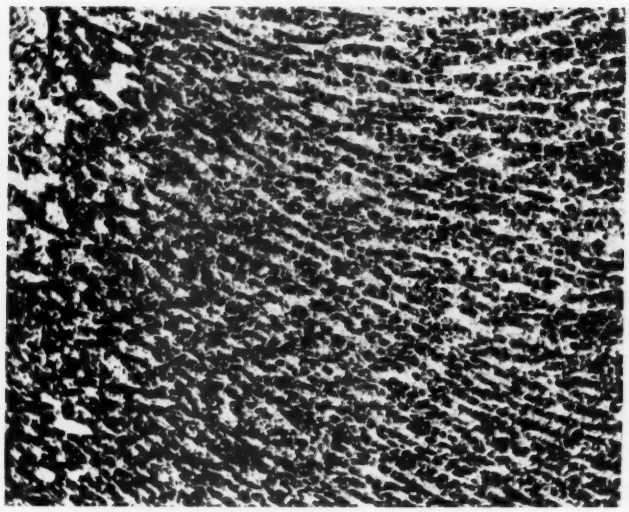

Fig. 3. — Photomicrograph showing a section of the suprarenal gland of a rat exposed to stress for 23 days. A broad necrotic zone extending over the entire cortex. Haemorrhages occurring in abundance. Cortico-medullary junction marked by arrow. ×200.

weight per 100 g. The figure arrived at as an average for the rats exposed to stress was 4.56 mg /100 g (compared to 3.62 mg/100 g for the control animals).

The test animals had undergone an increase in the number of beta cells, in some cases to a very considerable degree (Fig. 1). These cells could be noted in greatest abundance in the central part of the anterior lobe of the pituitary body. In the case of one rat, which had been exposed to stress for 105 days, the central part of the anterior lobe had become quite blue in color as a consequence of the great increase in beta cells. In some cases the number of cells had not increased so much but they did appear unusually large.

Generally, various signs of degeneration were evident in the beta cells. The nucleus tended to be shrunken and deformed. Distinct granules could not be detected; the cells appeared turbid.

Clear granules could not usually be distinguished in the cytoplasm of the alpha cells. No distinct degenerative changes could be detected in either these or the gamma cells.

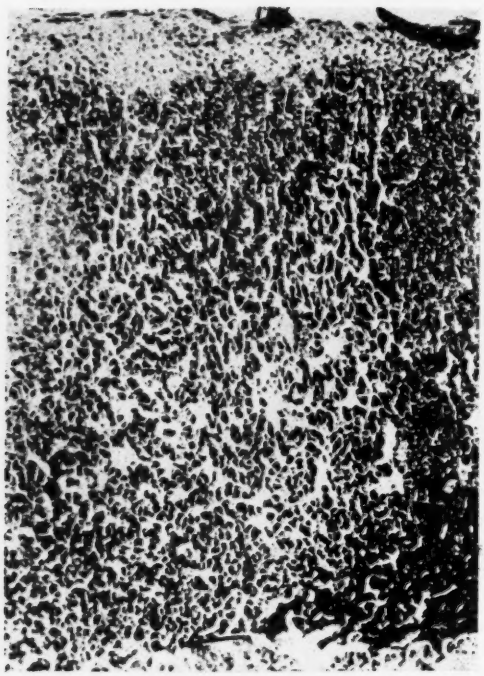

Fig. 4. — Photomicrograph showing a section of the suprarenal gland of a rat exposed to stress for 27 days. A broad necrotic zone extending over practically the entire cortex. Better preserved tissue on the right side. Zona glomerulosa visible at top as a light area. Its cells hypertrophic, their nuclei pycnotic. Cortico-medullary junction marked by arrow. × 150.

SUPRARENAL GLAND. — The suprarenal gland of the rats which had undergone stress was larger (20.4—15.3 mg) than that of the control animals (13.3—8.0 mg). In order to eliminate the influence of differences in the size of the animals, the ratio of the weight of the suprarenal gland to the weight of the animal per 100 g was calculated. The mean thereby arrived at (17.0 mg/100 g) is clearly greater than the corresponding figure (11.1 mg/100 g) for the control animals.

Cortex. — Strikingly great changes regularly occurred in the suprarenal bodies of the test animals. The zona glomerulosa could be seen in nearly all the preparations as a fairly light zone. The cells had swollen and taken on a blistery appearance, with the nuclei often pycnotic (Fig. 4). The most marked changes occurred

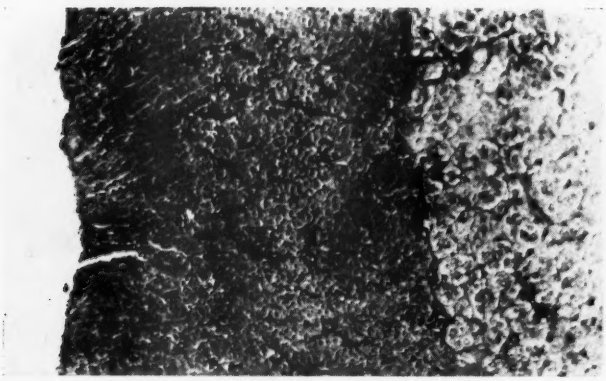

Fig. 5. — Photomicrograph showing a section of the suprarenal gland of a normal rat. Compare diameter of cortex to Figs. 4, 6 and 7, magnified to the same degree. ×150.

usually in the fascicular zone. Many of the test animals exhibited in places lesions with detached, necrotic cells.

In other test animals the entire fascicular zone showed grave lesions, which in places extended into the reticular zone. The cells were usually hypertrophic; vacuoles of varying size could be observed in them and various degenerative changes had taken place in the nuclei and the cytoplasm. The most serious lesions were found in two rats that had undergone 23 days of stress. (Fig. 2, 3). Hemorrhages had occurred in the cortex of the suprarenal glands of all the test animals.

Medulla. — Dilatation of the lumina of the veins in the medullae of the suprarenal glands appeared to have taken place. The cells of the medullae were hypertrophic and vacuolic changes tended to appear.

DISCUSSION

Comparing our results with those arrived at by Schunk and Cornelius, it is to be noted that in our experiments quite serious lesions generally developed in the rats' suprarenal glands, whereas the German researchers could not perceive any degenerative changes in the suprarenal glands of their test animals. The stresses we had

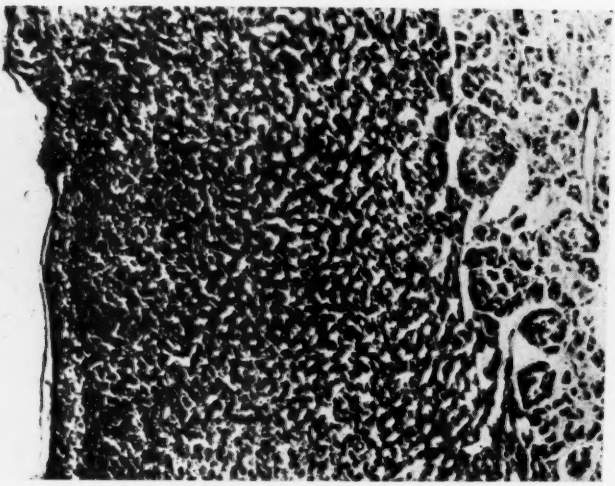

Fig. 6. — Photomicrograph showing a section of the suprarenal gland of a rat exposed to stress for 12 days. Worst lesions observable in fascicular zone. Dilated capillaries, haemorrhages and diffuse degeneration. Note also several hypertrophied light cells in zona glomerulosa. ×150.

arranged were applied for shorter periods of time, but evidently their effect was stronger.

In both investigations a marked increase in the beta cells of the pituitary body could be detected. These cells evidently have an important function in the defensive reactions of the organism against different harmful agents. In an earlier work (2) it was shown that large doses of insulin likewise cause enlargement as well as an increase in the number of beta cells.

It was astonishing to see how powerfully psychic stress affects rats. Most of them became fatigued and death often came after four weeks' treatment. In some cases pneumonic lesions could be detected in their lungs. Evidently, their resistance to infection had become considerably lowered, inasmuch as rats of the same stock and living under the same conditions, with the exception of the stresses, remained in good health.

Our observations cannot, of course, be applied to human beings as such; but it would appear obvious that protracted heavy psychic stress is bound to cause serious lesions in human beings too. Uotila and Pekkarinen e.g. have cited cases in which death has been

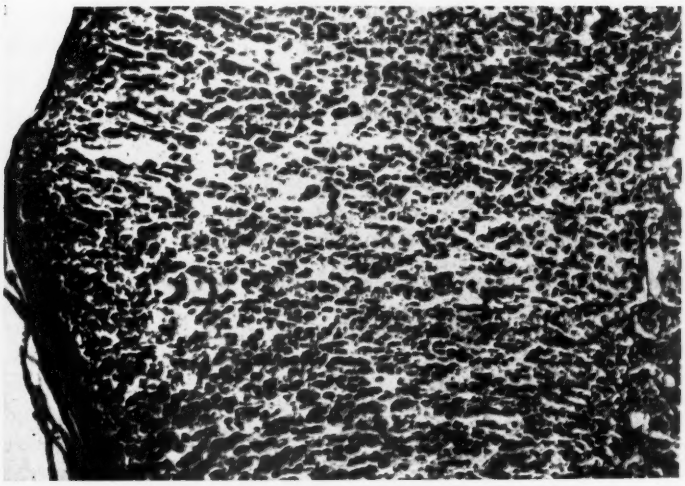

Fig. 7. — Photomicrograph showing a section of the suprarenal gland of a rat exposed to stress for 30 days. Note hypertrophy of cortex and subsequent necrosis. Borderline between cortex and medulla indistinct. ×150.

preceded by intensive stress causing hypertrophy of the cortical cells and a mottled appearance of the cortical tissue, owing to the fact that some cells are acidophilic and some had been vacuolated. In some of these cases the period of stress had been relatively short, but in those with a long period of reaction, lasting some weeks or even longer, lymphocytic infiltrates and even fibroses were observed.

SUMMARY

The effect of psychic stress on the cells of the pituitary body and the suprarenal glands of white rats was studied. Conditions of stress were maintained over periods ranging from 12 to 105 days.

1) Stress powerfully affected the general condition of the rats and evidently lowered their resistance to infection.

2) Under stress the pituitary body of the rats possibly became slightly enlarged. An increase in the number of beta cells was noted in the pituitary body; in some cases the increase was remarkably large. The cells often varied in size and in general no clear granules could be seen. The cells often showed signs of degeneration. As for the alpha cells, neither clear granules nor degeneration could be noted.

3) Under stress the rats' suprarenal glands clearly increased in size. Grave changes occurred in the cortex of these glands, notably in the fascicular zone. The cells were usually hypertrophic throughout the cortex. Necrotic cells often appeared in a broad zone across the entire cortex.

In the medulla of the suprarenal glands the lumina of the veins were often dilated, the cells of the medulla usually being hypertrophied and vacuolated.

The lesions caused by psychic stress are thus astonishingly grave, especially in the cortex of the suprarenal gland.

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FROM THE HOSPITAL OF THE FOUNDATION FOR RHEUMATIC DISEASES, HEINOLA

SERUM PRECIPITATION TEST IN RHEUMATOID ARTHRITIS ACCORDING TO JOKINEN

by

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The diagnosis of rheumatoid arthritis may be difficult at all stages of the disease owing to considerable variations in its clinical nature, which is often atypic (3, 9). Therefore, a rheuma-specific blood reaction would be of the greatest significance both for the diagnosis of the disease and for treatment. However, such a reaction has not yet been found.

Percentally some of the non-specific rheumatism reactions give remarkably positive results. According to Wager's investigations (17), the Waaler-Rose reaction in rheumatoid arthritis is positive in 61 per cent of the cases, in rheumatic fever in 3 per cent only. In patients suffering from other diseases and also in the healthy individual, positive results vary between 1 and 2 per cent. Many investigators regard Weltmann's coagulation reaction as an excellent indicator of prognosis in rheumatoid arthritis (15, 18). C-reactive proteins have been found to appear in some active cases of the disease (1, 2).

Jokinen (7), with his serum reaction, has obtained a positive result in 71 per cent in rheumatic diseases (6), there being a positive reaction in 71 per cent of the 55 cases of rheumatoid arthritis investigated. Healthy subjects reacted 100 per cent negatively. In rheumatic diseases the reaction is non-specific.

At the Rheumatic Foundation Hospital, Heinola, we studied 228 clinically clear cases of rheumatoid arthritis by means of the Jokinen reaction and obtained the afore-mentioned percentally very differing values.

TECHNIQUE

2.5 ml of 94 per cent ethyl alcohol was measured into a test tube with an inside diameter of 11—12 mm. 0.25 ml of serum was pipetted into it dropwise at room temperature without the drops touching the sides of the tube. The tube was placed, without shaking, in a refrigerator at +4° C, where it was allowed to stand unsealed for 24 hours. It was then centrifuged at 2,500 r.p.m. for 5 minutes in an angle head centrifuge, the test tube at an angle of 45 to the rotation plane, the rotation radius was 10 cm. The supernatant was discarded, and the tube stood upside down at room temperature for 15 min. One ml of 95 per cent sulphuric acid was now added to the precipitate and allowed to act for 5 min. The reading was taken.

The result was considered negative (—) when the precipitate remained completely undissolved. In such a case the sulphuric acid remained colourless and did not warm up. The more positive the result, the more the precipitate dissolved, the hotter the sulphuric acid grew, and the darker drown it became. The result was taken as weakly positive (±) if some of the precipitate clearly dissolved and a weak brown colour developed, and strongly positive (+) if the precipitate was completely dissolved and a strong brown colour formed. In some cases Jokinen used the sign (—?). In such cases the result was regarded as negative, even if bordering an a weakly positive reaction (±). We have considered these results (—?) negative because some normal sera react in this fashion. This has been confirmed in blank control tests with Jokinen. On all serums the tests were carried out on two successive days and the average of the results obtained was taken as the final reading.

MATERIAL

The 228 unselected cases of rheumatoid arthritis can, according to the generally accepted classification (American Rheumatism Association), be divided into four different groups, on the basis of the clinical and roentgenological picture of the disease. The first group (stage I) consisted of 40 cases, the second (st. II) of 101, the third (st. III) of 60 and the fourth (st. IV) of 27.

RESULTS

When the results of the different stages of the disease are compared to one another, it seems likely that the amount of cases giving a positive reaction is directly proportional to the degree of clinical severity of the disease (Table 1).

According to Jokinen's investigations (6, 7), the reaction results are divided in proportion to the rate of erythrocyte sedimentation,

TABLE 1

THE EFFECT OF STAGE OF THE DISEASE ON THE RESULTS

Material	Cases	Positive		Negative	
		Number	Per Cent	Number	Per Cent
Examined cases ..	228	32	15,4	196	84,6
Stage of disease I	40	4	10,0	36	90,0
II	101	12	11,9	89	88,9
III	60	10	16,7	50	83,3
IV	27	6	22,2	21	77,8

so that when the ESR is under 20, all the sera react negatively. When the ESR values rise above 20, the number of positive results also rises, so that with an ESR of over 45 all cases react positively. Later, however, Jokinen found that in cases where the ESR is high, the positive reaction comes forth more clearly in rheumatic fever than in rheumatoid arthritis. In our material a positive reaction appeared relatively more frequently in the ESR group over 100, in fact, in 88.9 per cent on the cases (Table 2).

Jokinen discovered (6) that in feverish cases the serums investigated by him were 100 per cent positive and all negative cases were without fever, but fever had no clear correlation to the appearance of a positive reaction. Our series included 83 feverish cases, 82 per cent of which reacted negatively (Table 3).

Jokinen and Kaipainen (8) analysed 20 serum proteins electrophoretically and ascertained that when the globulins were increased and the albumins decreased, the reaction generally became positive.

TABLE 2

THE CORRELATION OF THE SEDIMENTATION RATE ON THE RESULTS

Sedimentation Rate	Number	
	Positive	Negative
—20	1	64
20—30	0	34
31—45	5	41
46—100	18	56
101—	8	1
Total	32	196

TABLE 3

THE CORRELATION OF FEVER ON THE RESULTS

	Cases	Positive	Negative
Without fever	145	17	128
With fever	83 228	15	68
Total ..		32	196

Of these cases five were those of rheumatoid arthritis. Our series included 32 positive reactions. From these cases we determined the albumins and globulins by precipitation (4). The results are set out in Table 4, from which it is evident that in the greatest part of the positively reacting serums the alb./globul. was abnormal, although positivity does not probably follow linearly the proportional rise of the globulins at all. In addition, we also determined from 60 unselected negatively reacting sera the albumins in relation to the globulins and found that a negative reaction may appear in a case of a pathological protein relationship (Table 5).

According to Jokinen's study, cortisone treatment weakens the positivity of the reaction (5). In our series 85 patients had received this treatment and ACTH treatment in addition. These reacted 17.6 per cent positively, while the other 143 gave a positive reaction in 11.6 per cent cases. We noticed the changing of the reaction from positive to negative in the following two cases: Case No. 1 — two years before the test the patient had had many months of cortisone treatment and the reaction was positive. After the test had been performed, the patient was given 130 mg of hydrocortisone (Cortef) per os, and ten days after terminating the treatment the serum specimen reacted negatively. Case No. 2 — for ten days before the test the patient had been given 100 units of ACTH (Acton prolongatum) and the reaction was positive (±), but when the treatment was continued the reaction turned negative. It seems likely likely that ACTH and hydrocortisone effect the positive reaction, rendering it negative, but the effect may be short lived.

Apparently gold treatment does not effect the reaction. 185 of the cases investigated had received such treatment and these, 27 reacted positively.

In positively reacting cases the average age in our experiment was 40.6, in negatively 36.2.

Jokinen found that the duration of the disease does not effect the reaction results (6), a fact that also emerges from out cases. The positively reacting cases had the disease for an average period of 7.9 years, the negatively reacting for 7.7 years.

Jokinen (7) has found a positive reaction in 10 cases out of 13 in the diseases of the liver parenchyma. We studied the significance of the reaction as a liver function test in 60 cases of rheumatoid arthritis. Together with Jokinen's precipitation test, the following

TABLE 4

COMPARISON OF 32 CASES GIVING POSITIVE RESULTS AND SERUM PROTEINS BY »JOKINEN'S SERUM PRECIPITATION TEST«

Case No.	»Jokinen's Serum Precipitation Test« Final Reading	Total Protein	Albumin	Globulin	Alb./Glob.
1	±	8.4	5.4	3.0	1.80
2	±	8.0	5.1	2.9	1.75
3	±	8.2	5.2	3.0	1.73
4	±	8.0	4.8	3.2	1.50
5	±	7.7	4.4	3.3	1.33
6	±	7.7	4.4	3.3	1.33
7	±	8.0	4.5	3.5	1.29
8	±	7.5	4.2	3.3	1.27
9	±	8.2	4.5	3.7	1.22
10	±	8.2	4.5	3.7	1.22
11	±	7.2	3.9	3.3	1.18
12	±	7.2	3.9	3.3	1.18
13	±	8.5	4.5	4.0	1.12
14	±	8.5	4.5	4.0	1.12
15	±	8.2	4.3	3.9	1.10
16	±	9.0	4.7	4.3	1.09
17	±	8.4	4.2	4.2	1.00
18	±	8.4	4.2	4.2	1.00
19	±	7.8	3.9	3.9	1.00
20	±	8.4	4.2	4.2	1.00
21	±	7.1	3.5	3.6	0.97
22	±	8.6	4.1	4.5	0.91
23	±	7.1	3.3	3.8	0.87
24	±	6.9	3.2	3.7	0.86
25	+	8.7	4.7	4.0	1.18
26	+	8.8	4.7	4.1	1.14
27	+	8.2	4.2	4.0	1.05
28	+	7.9	3.7	4.2	0.88
29	+	10.2	4.7	5.5	0.85
30	+	9.9	4.4	5.5	0.80
31	+	8.5	3.7	4.8	0.77
32	+	9.9	3.0	6.9	0.45

TABLE 5

THE ALBUMIN GLOBULIN RATIO OF 60 UNSELECTED CASES GIVING NEGATIVE RESULTS BY »JOKINEN'S SERUM PRECIPITATION TEST«

Negative Results	Alb./Glob. > 1.70	Alb./Glob. < 1.70 and 1.70	Alb./Glob. < 1.12
60	17	43	6

investigations were made: serum bilirubin determination (13), thymol test (11), Takata-Ara (12), Kunkel (10), galactose tolerance test (16) and hippuric acid synthesis test (14). The galactose tolerance test was positive in 30 cases, but of these only 4 reacted positively in the Jokinen's test. The corresponding figures in the hippuric acid test were 18 and 3. Pathologic bilirubin values were found in 4 cases, all of which reacted negatively in the Jokinen's test. On the basis of this, it seems likely that there is no direct correlation between the so called liver function tests and a positive Jokinen's test in cases of rheumatoid arthritis.

SUMMARY

1. Sera from 228 accurately diagnosed cases of rheumatoid arthritis were tested by using Jokinen's serum precipitation test; a positive result was obtained in only 15.4 per cent of cases. This suggests that Jokinen's test has no diagnostic significance in rheumatoid arthritis cases.

2. Clinically, the most severe cases gave positive reactions more frequently than the milder cases.

3. Occurrence of the positive mode of reaction has no correlation to the fever.

4. Sera with a very high sedimentation rate have been found to give positive reactions more frequently than those with a low sedimentation rate.

5. Sera with a low albumin globulin ratio gave positive reactions proportionally more frequently than negative reactions.

6. ACTH, cortisone and hydrocortisone treatments appear to change the mode of reaction towards the negative.

7. In cases of rheumatoid arthritis there was no direct correlation between the so called liver function tests and the Jokinen's serum precipitation test.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY,
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STUDIES OF THE USE OF 2, 3, 5, TRIPHENYLTETRAZOLIUM CHLORIDE IN TUBERCLE BACILLI SENSITIVITY TESTS

by

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Since R. Kuhn and D. Jerchel (5) in 1941 found that living bacteria are capable of reducing tetrazolium salts to coloured compounds, the use of these salts in the different fields of bacteriology has been subjected to relatively extensive research.

The tetrazolium salts have also been used (1, 2, 6, 8) in research concerned with the viability of tubercle bacilli and a new sensitivity determination method has been developed (7). The ability of the bacilli to reduce tetrazolium salts, however, does not permit definite conclusions concerning their viability; it merely reflects the activity of the dehydrogenases (3).

The object of the present work is to study the use of 2, 3, 5, triphenyltetrazolium for sensitivity determinations of tubercle bacilli, first and foremost via-à-vis streptomycin.

MATERIAL

H37Rv was chosen as the tubercle bacillus strain for the tests. The culture media used were: (1) Fisher's modification of Dubos's liquid culture medium (4). Tween 80 the authors replaced with oleic

acid when a relatively even coarse-grained bacillus suspension was obtained. (2) Löwenstein-Jensen's solid culture medium. The tuberculostatic drugs used were dihydrostreptomycin (Specia Paris) and isoniazide (Hoffman La Roche). The strength of the 2, 3, 5, triphenyltetrazolium solutions was 0.5% and 1%. Distilled water, Sauton's, Kirchner's and Dubos's liquid media were used to dissolve streptomycin and isoniazide.

METHODS AND RESULTS

The effect of dihydrostreptomycin on tubercle bacilli was first studied in Fisher's culture medium: 0.5 ml of homogeneous H37Rv suspension, 24 days old, was pipetted into test tubes containing 2 ml of the medium. The final bacillus concentration obtained was 2 mg per ml of culture medium. The weight of the bacilli was checked in a Hopkins vaccine tube. Prior to this a 2 mg bacillus dose had been established empirically as the most suitable for these conditions; smaller doses gave too faint a colour and larger masses of bacilli retained their reducing ability. The number of bacilli was obviously too great in the last-mentioned case for the total quantity of streptomycin. Dihydrostreptomycin was added to the test tubes giving the following concentrations: 100, 10, 1 and 0 γ per ml of culture medium. The tubes were placed in an incubator at 37° C. 2 days later the tubes were examined daily for streptomycin effect by adding 0.3 ml of 0.5% tetrazolium solution and then returning the suspensions to the incubator for 2 hours. The coloration of the sedimented bacilli was noted.

The inhibition of the reduction induced by dihydrostreptomycin was visible on the 3rd day. The results can also be evaluated quantitatively by extracting the red colour from the bacilli with different alcohol solutions and by measuring the colour of the extracts with a colorimeter. The standard curve compiled for this purpose showed a linear rise in the absorption when the weight of the mass of bacilli increased. The results obtained correspond approximately to those of H. Mac Vandiviere et al.(7).

The effect of streptomycin on tubercle bacilli was apparent fairly rapidly with the method described above. The writers consider, however, that this method, which presupposes homogeneous

The results obtained will be clear from the following table.

TABLE

Concentration Streptomycin γ/ml	Days from Commencement of Incubation									
	1	2	3	4	5	6	7	8	9	10
0		+++	+++	+++	+++	+++	+++	+++	+++	+++
0		+++	+++	+++	+++	+++	+++	+++	+++	+++
0		+++	+++	+++	+++	+++	+++	+++	+++	+++
1		++	—	—	—	—	—	—	—	—
1		++	—	—	—	—	—	—	—	—
1		++	—	—	—	—	—	—	—	—
10		++	—	—	—	—	—	—	—	—
10		++	—	—	—	—	—	—	—	—
10		++	—	—	—	—	—	—	—	—
100		+	—	—	—	—	—	—	—	—
100		+	—	—	—	—	—	—	—	—
100		+	—	—	—	—	—	—	—	—

+++ = strong, ++ = faint, + = very faint colour, — = colourless solution

bacillus suspensions of a certain strength, is too laborious for routine purposes. They therefore abandoned further experimentation with a liquid culture medium.

In order to study the effect of antibiotics on colonies growing on a solid culture medium 1 γ of bacillus strain H37Rv was inoculated into test tubes containing Löwenstein-Jensen culture medium. 3 weeks later, when numerous small colonies were visible, 2 ml of tuberculostatic substances dissolved in distilled water, Sauton's, Kirchner's and Dubos's culture medium were pipetted into each tube. The concentrations were as follows: dihydrostreptomycin 1000, 100, 10, 1 and 0 γ per ml; isoniazide 1,0; 0.2 and 0 γ per ml. The combination 10 γ/ml streptomycin and 1 γ/ml isoniazid was also used. After this the tubes were incubated at 37° C in an oblique position. Readings were taken on the 3rd, 6th, 9th and 12th day from the commencement of incubation by adding 0.5 ml of 1% tetrazolium solution into all the tubes and keeping them a further 2 hours at 37° C. 4—5 tubes of each drug concentration were used for each reading.

In the tests performed with a solid culture medium reduction was established in all tubes including the isoniazide-streptomycin combinations, even as late as the last day of reading, i.e. the 12th day from the commencement of incubation. No absolute correlation was observed between the degrees of reduction and the concentration of antibiotics, though there was some tendency in the larger concentrations towards a reduction weaker than in the control tubes without antibiotics. Moreover, the control tubes also, i.e. tubes without tuberculostatic substances, stained unevenly and several colonies remained rather faint in colour. The intensity of the colour was strongest in the tubes in which the antibiotic was dissolved in Dubos's culture medium containing Tween 80.

The fact that the reducing ability of bacilli in liquid culture medium disappeared in certain conditions under the influence of streptomycin but did not do so in colonies on a solid culture medium, may be due to a better antibiotic effect in liquid cultures and failure of the drug to reach the bacilli equally well in the colonies on the solid culture medium. In addition, unknown factors maintaining a dehydrogenase system may perhaps also contribute to the phenomenon.

SUMMARY

Tubercle bacilli in liquid culture medium lost their ability to reduce triphenyltetrazolium 3 days after the addition of an antibiotic. Tuberculostatic substances added to colonies grown on a solid culture medium were fairly ineffective.

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FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF HELSINKI

EVALUATION OF THE ADRENOLYTIC POTENCY OF ERGOT ALKALOIDS BY USING THE TERMINAL ILEUM OF THE GUINEA PIG AS A TEST OBJECT

by

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(Received for publication October 15, 1954)

Both biological, chemical and physical methods have been used for the evaluation of ergot alkaloids. The colorimetric and fluorescence methods are, however, considerably less sensitive than the biological ones, being moreover less specific as they are based on the lysergic acid component of the alkaloid. It has not been possible so far to distinguish colorimetrically the sympatholytic ergot alkaloids from the oxytocic substances of the ergometrine group (9). The biological methods, the guinea pig seminal vesicle method (8) and the rabbit's uterus method (1) being those most commonly used, have proved to be extremely sensitive and specific. According to Rothlin and Brügger (9), however, the values obtained by these biological methods for the relative potency of the sympatholytic drugs vary greatly. *E.g.*, measured with the isolated seminal vesicle of the guinea pig ergocornine proved to be twice, ergocristine and ergokryptine four times more effective than ergotamine. Measured with the isolated rabbit uterus, on the other hand, ergocornine was 50 per cent less effective than ergotamine, ergocristine and ergotamine being approximately equally effective. Ergokryptine only was about 50 per cent more effective than ergotamine. Also to the hydrogenated ergot alkaloids the rabbit's uterus was considerably less sensitive than the seminal

vesicle of the guinea pig. The potency of the hydrogenated ergot alkaloids was 2.25—5.0 times higher than that of ergotamine, when the experiments were made with the rabbit's isolated uterus. When the evaluation was made with the seminal vesicle of the guinea pig, the hydrogenated alkaloids were 7—35 times more effective than ergotamine.

It seemed therefore to be of interest to study the behaviour of the terminal ileum of the guinea pig toward these changes in an ergot alkaloid molecule. Viz., its longitudinal muscle layer responds to adrenaline and noradrenaline just as does the smooth muscle of the seminal vesicle, i.e. by contraction (5, 6). It also seemed to be worth while to study to what extent this usually wasted organ could be used for the pharmacological technique, especially for the assay of sympatholytic drugs.

METHOD

Adult guinea pigs, most of which were male, were used. The animals had an oat-water diet for two to three days and fasted entirely for one day before being killed. The animal was killed by a blow on the head and by cutting off the neck blood vessels. The abdomen was opened and the ileum was cut close to the caecum. An about 10 cm long portion was removed from its terminal end, and it was cleaned from the mesenteric tissue and its contents were washed with Tyrode's solution if so needed. Two about 2.5 cm long segments were cut from the distal end of the ileum portion, they were attached to the oxygen tubes and placed in the tissue chambers of the thermostat in the oxygenated Tyrode's solution at the temperature ca. 34°C. The segments were allowed to recover without tension at this temperature for 15 minutes, then their upper end was attached to the lever of the recorder with a strip. Palmer's simple lever with all-metal frontal writing point was used. The preparation was kept under slight draught for 45 minutes before the stimulation with adrenaline began.

The extensive relaxation of the ileum was found to be necessary, therefore an attempt was made to eliminate the factors increasing the tonus. For this purpose the composition of Tyrode's solution was changed so that glucose and sodium phosphate were left out and the pH of the solution, being usually 8.05, was lowered to 7.35 by diminishing the amount of sodium bicarbonate into one-fifth of the usual. Tyrode's solution was thus of the following composition: 0.8 per cent NaCl, 0.02 per cent KCl, 0.02 per cent $CaCl_2$, 0.01 per cent $MgCl_2$, and 0.02 per cent $NaHCO_3$. The change in the composition somewhat diminished the sensitivity of the ileum to adrenaline, but it increased considerably its sensitivity to the adrenolytic effect of sympatholytic drugs. A sufficient inhibition of about 50 per cent was thus induced by very small alkaloid concentrations; an important point

when studying the potency of ergot alkaloids, whose inhibitory action slowly subsides. The ileum recovers then more quickly and endures better the strain of a prolonged test in any case.

Adrenaline doses were added by means of a 1.0 ml tuberculin syringe, at 20 minutes intervals. After the intestine was stabilized the blocking agent was added one minute after the adrenaline-induced contraction and was thus allowed to act on the preparation for 19 minutes. Adrenaline was added in the presence of the antagonist. After the action of adrenaline, the ileum segment recovered rather completely in 10—15 minutes, being, however, capable to respond to adrenaline almost unchanged even longer. Tyrode's solution was changed six times to facilitate the recovery of the intestine after the adrenaline stimulations. The tests were performed at the constant temperature between 36°C and 30°C, usually at 34°C. The volume of the tissue chamber was six ml.

The adrenaline solution of 1: 1,000 was prepared as follows: Five mg of crystalline adrenaline base were dissolved in 0.5 ml of 0.1 N HCl and 4.5 ml of distilled water were added. The required dilutions of this stock solution were made by using 0.9 per cent NaCl solution. The commercial adrenaline ampuls available in markets can also be used. The solutions of ergot alkaloids were prepared by diluting the contents of Gynergen, Dihydroergotamine and Hydergine ampuls (Sandoz) with 0.9 per cent NaCl solution, the concentrations being those of alkaloid salts. The dilutions were freshly prepared for each test. The ergocristine solution was prepared from the crystallized ergocristine (Sandoz) according to Brügger (2).

RESULTS AND DISCUSSION

Response of the Terminal Ileum of the Guinea Pig to Adrenaline.— According to Munro (5, 6), the terminal ileum of the guinea pig responds to adrenaline by contraction in length of about 9—15 cm. The tonus of the ileum preparation being moderate, *i.e.*, when the usual Tyrode's solution with an addition of glucose of 1: 10,000 or more is used at the temperature of 36°—37° C, very small adrenaline concentrations (1: 1,000,000,000—1: 50,000,000) can induce relaxation; while the contraction is produced only with greater concentrations (Fig. 1). It appears that adrenaline has greater affinity to inhibitory than to excitor receptors, and that it is not until the inhibitory receptors have been saturated with adrenaline that the action of adrenaline is directed to the excitor receptors. Similar finding was observed by Munro (7) in an ileum segment and by Rothlin and Brügger (9) in the rabbit's isolated uterus. In this respect the terminal ileum of the guinea pig differs from the seminal vesicle, upon which adrenaline has no relaxing effect (2).

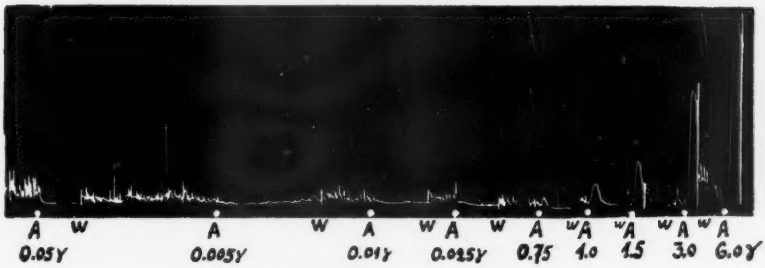


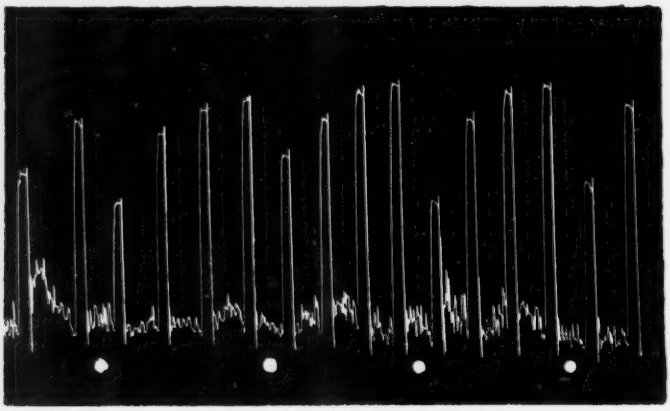
Fig. 1. — The relaxation of the isolated terminal ileum of the guinea pig in the small and the contraction in greater adrenaline concentrations in the usual Tyrode's solution containing 0.01 per cent of glucose. The doses of adrenaline (A) were given at 20 minutes intervals. Temperature was 36° C. W = washing.

The motor effect of adrenaline on the terminal ileum of the guinea pig is obviously sympathomimetic in nature since all the most common sympatholytic drugs showed inhibitory activity against it in very small concentrations. On the other hand, atropine, antihistaminic and ganglion-blocking agents, such as hexamethonium bromide and Pendiomid, had no action on the motor effect of adrenaline in the corresponding concentrations.

Behaviour of the Terminal Ileum of the Guinea Pig to the Ergot Alkaloids. — When the usual Tyrode's solution was used, the sensitivity of the terminal ileum to the ergot alkaloids was approximately similar to that of the seminal vesicle. In the concentration of 1: 60—70,000,000 ergotamine tartrate produced about 50 per cent or slightly greater inhibition of the contraction induced by adrenaline. In the concentration of 1: 500,000,000 dihydroergotamine methansulphonate produced similar inhibition. The reported modification of Tyrode's solution increased the sensitivity of the terminal ileum to ergot alkaloids almost twofold, so that ergotamine tartrate in the concentration of 1: 125,000,000, dihydroergotamine methansulphonate in the concentration of 1: 1,000,000,000, and Hydergine in the concentration of 1: 2,500,000,000 produced a decrease of about 50 per cent in the effect of adrenaline. Among the test objects known, the terminal ileum of the guinea pig seems to be most sensitive to the ergot alkaloids.

The rather slow reversibility is characteristic of the adrenolytic effect of ergot alkaloids (9, 4). The stronger the alkaloid concentra-

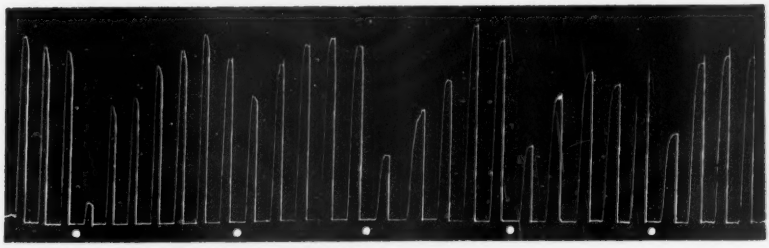
tion and the longer its effect, the slower the restoration of the responsiveness of the ileum preparation to adrenaline. The effect of Hydergine, especially, is very slowly reversible or partly irreversible in larger concentrations. The effect of the alkaloid concentrations, which produce ca. 50 per cent or slightly greater inhibition in the adrenaline effect is, however, rather reversible in 1.5—2 hours, provided that the ileum preparation is not otherwise tired.



E 1 1: 70 mill. H 1 1: 1,050 mill. H 2 1: 700 mill. E 2 1: 70 mill.

Fig. 2. — The responses of the isolated terminal ileum of the guinea pig to adrenaline and ergot alkaloids in the usual Tyrode's solution containing 0.01 per cent of glucose at 36° C. The doses of adrenaline were given at 20 minutes intervals. The first contraction was induced by 2 μg, the others by 3 μg of adrenaline (1: 2,000,000).

Fig. 2 shows an assay, in which the usual Tyrode's solution containing 0.01 per cent of glucose was used. The temperature was 36° C. The spontaneous movements of the ileum segment were prominent and the basic line proved to be unlinear. In the concentration of 1: 70,000,000 ergotamine showed inhibition of 41 per cent in the first trial and 34 per cent in the last. Hydergine showed inhibitory action of 23 per cent in the concentration of 1: 1,050,000,000 and 46 per cent in the concentration of 1: 700,000,000. Fig. 3 shows a test in which the modified Tyrode's solution (pH 7.35) at the temperature of 34° C was used. Spontaneous movements did not occur and the basic line remained



	E 1	H 1	E 2	H 2	E 3
	1: 100 mill.	1: 3,000 mill.	1: 120 mill.	1: 2,400 mill.	1: 150 mill.
Inhibition	88 %	23 %	63 %	58 %	50 %

Fig. 3. — The evaluation of the potency ratio of Hydergine to that of ergotamine by means of the isolated terminal ileum of the guinea pig. Tyrode's solution modified by the author was used. Temperature was 34° C. All contractions were produced by 3 μg of adrenaline (= 1: 2,000,000) at 20 minutes intervals. E = ergotamine doses, H = Hydergine doses.

linear during the whole test. In the concentration of 1: 150,000,000 ergotamine showed inhibition of 50 per cent, and Hydergine 58 per cent in the concentration of 1: 2,400,000,000. The terminal ileum retained its responsiveness to adrenaline almost unreduced for more than nine hours in this test.

Relative Potency of Some Ergot Alkaloids. — Ergotamine tartrate was used as a standard. The comparison of the adrenolytic potency of the alkaloid with that of ergotamine was performed so that a certain number of comparative assays were made on each of the concentration ratios of ergotamine and the alkaloid, different ileum preparations being used in each trial. The concentration ratio, in which the adrenolytic potency of the standard and the compared alkaloid was equally strong in most tests, indicated the relative potency of the substance concerned as compared with the potency of ergotamine. The potency of the adrenolytic effect is given as a percentage that is obtained by counting how many per cent the ileum contraction (the recorded height in mm), induced by the same adrenaline dose after the sympatholytic drug was allowed to act on the ileum preparation for 19 minutes, was diminished from what it was before the addition of the sympatholytic drug. The adrenolytic effects were considered equally strong when the difference of the percentages was less than ten. *E.g.*, in the test illustrated in Fig. 3, ergotamine's blocking action of 63 per cent in the concentration of 1: 120,000,000 was considered stronger than the 50 per cent

TABLE 1

COMPARISON OF ADRENOLYTIC POTENCY OF SOME ERGOT ALKALOIDS

Alkaloids Compared with Each Other	Concentration Ratio	Number of Trials	Adrenolytic Action of Drugs			Relative Potency (Ergotamine=1)
			Less Potent than the Standard	As Potent as the Standard	More Potent than the Standard	
Ergotamine / DHE	6 : 1	6	0	1	5	
	7 : 1	6	0	4	2	
	8 : 1	6	2	4	0	8
	10 : 1	6	6	0	0	
Ergotamine / Hydergine	6 : 1	8	0	1	7	
	8 : 1	4	0	2	2	
	10 : 1	6	2	2	2	
	12 : 1	7	2	3	2	
	15 : 1	6	2	4	0	
	20 : 1	6	1	5	0	20
	25 : 1	3	1	2	0	
	30 : 1	6	5	1	0	
Ergotamine / Ergocristine	3 : 1	2	0	0	2	
	4 : 1	2	0	2	0	4
	5 : 1	2	1	1	0	

inhibition in the concentration of 1: 150,000,000. On the other hand, the 58 per cent inhibition by Hydergine in the concentration of 1: 2,400,000,000 was considered as strong as the inhibition induced by ergotamine in the concentration of 1: 120,000,000.

Table 1 shows that dihydroergotamine was eight, Hydergine 20, and ergocristine four times more effective than ergotamine.

Behaviour of the Terminal Ileum of the Guinea Pig to Some Changes of the Ergot Alkaloid Molecule. — Brügger (2) and Rothlin and Brügger (9) have demonstrated that on the isolated seminal vesicle of the guinea pig ergocristine inhibits the adrenaline effect four times stronger than ergotamine, while both the alkaloids are approximately as strong on the rabbit's isolated uterus. On the isolated terminal ileum of the guinea pig the adrenalytic action of ergocristine is about four times more effective than that of ergotamine. Accordingly, the isolated terminal ileum behaves to the

dimethylation of the keto acid component of the ergot alkaloid molecule, both qualitatively and quantitatively, as does the isolated seminal vesicle of the guinea pig; while both the organs of the guinea pig clearly differ from the rabbit's uterus in this respect.

Rothlin and Brügger (9) observed that the saturation of the double linking of the lysergic acid radical markedly increases the adrenolytic activity of all the natural ergot alkaloids. On the isolated seminal vesicle of the guinea pig the potency of the hydrogenated alkaloids was 7—12.5 times and on the rabbit's isolated uterus only 2.25—5.0 times higher than that of the corresponding natural ergot alkaloids. The seminal vesicle proved thus to be about 2.5—3 times more sensitive to the influence of hydrogenation than the rabbit's uterus. On the isolated terminal ileum of the guinea pig the adrenolytic action of dihydroergotamine was about eight times more effective than that of the corresponding non-hydrogenated alkaloid, ergotamine. The terminal ileum segment of the guinea pig thus behaves toward the hydrogenation of the ergotamine molecule as does the seminal vesicle.

Unfortunately, the hydrogenated derivatives of the alkaloids of the ergotoxine group were not available in separate compounds, only in the form of the combination product, Hydergine. Hydergine proved to be 20 times more effective than ergotamine in the isolated terminal ileum of the guinea pig. According to Brügger (2), DH-ergocornine was 25 times, DH-ergocristine 35 times, and DH-ergokryptine likewise 35 times more effective than ergotamine when evaluated in the isolated seminal vesicle of the guinea pig. Since Hydergine contains equal amounts of each three DH-alkaloids it ought to be approximately 32 times more effective than ergotamine. When performing the assays on the ileum segment and the seminal vesicle of the same guinea pig individual simultaneously, Hydergine proved to be equally effective in both the test objects. The isolated terminal ileum of the guinea pig thus behaves toward the dimethylation of the pyruvic acid component and to the hydrogenation of the lysergic acid component of the ergot alkaloid molecule as does the isolated seminal vesicle of the guinea pig. This behaviour suggests the similarity of the receptors concerned in these organs of the guinea pig.

Leitch and co-workers (4) reported that in the rat's isolated seminal vesicle Hydergine was on an average 1.64 times more

effective than ergotamine. Accordingly, the rat's seminal vesicle seems to be even less sensitive to the hydrogenation of ergot alkaloids than the rabbit's uterus, in which the average potency ratio of the single components of Hydergine to that of ergotamine was 3.7 as reported by Rothlin and Brügger (9). These potency values are not, however, entirely comparable, since the time of the action of alkaloids on the isolated organs were very different in the methods of Leitch and co-workers and of Rothlin (1 and 20 minutes, respectively). The maximal adrenolytic action of ergot alkaloids develops rather slowly (3, 9). Further assays are needed to elucidate the question whether the different duration of action of the sympatholytic drugs influenced the fact that piperoxane hydrochloride and ergotamine methanesulphonate had the same potency on the rat's isolated seminal vesicle (4), whereas on the isolated terminal ileum of the guinea pig piperoxane was considerably less effective than ergotamine (10).

SUMMARY

1. The method for the evaluation of the adrenolytic potency of sympatholytic drugs is described. The isolated terminal ileum of the guinea pig was used as a test object.

2. The method proved to be twice more sensitive to the sympatholytic ergot alkaloids than the guinea pig seminal vesicle method.

3. The following values were obtained for the relative potency of some ergot alkaloids: ergotamine 1, ergocristine 4, dihydroergotamine 8, and Hydergine 20.

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STUDIES OF THE MECHANISM OF THE ANOXIC SUPPRESSION OF EXTERNAL PANCREATIC SECRETION

by

K. J. V. HARTIALA

(Received for publication October 22, 1954)

In a previous paper (5) it was shown that the humorally stimulated pancreatic secretion is greatly depressed by anoxic anoxia. This reduction was considered to be at least partially a consequence of increased adrenal activity under the experimental conditions. Such activity is considered to manifest itself also during anoxia-type of stress. (2, 4, 6, 7, 9, 12). It is also known that the pancreas is sensitive to adrenaline action, which causes a prolonged vasoconstriction in the blood vessels supplying the gland and leads to a reduction in its secretion (1, 3, 10, 11).

In connection with the earlier studies attempts were made to elucidate the possible relationship between the anoxic suppression ot the pancreatic secretion and adrenal activity. The results of these experiments will now be reported.

MATERIAL AND METHODS

The experiments were carried on 3 mongrel dogs under light sodium pentobarbital anesthesia. The main pancreatic duct was cannulated and the pancreatic juice collected directly into calibrated tubes. Only the amount of juice was considered; no analyses were made of its constituents. A constant and continuous stimulus for the pancreas was employed. This was achieved by the injection of a secretin-pancreozymin solution into the femoral vein with a constant infusion pump. The rate of infusion was fixed to keep

the control pancreatic secretion at approximately half its maximal capacity. This was first determined by introducing a high dose of secretin.[1]

The anoxia effect was produced by having the animal breathe from a Douglas sack containing an 8 per cent oxygen 92 per cent nitrogen mixture. The expired air was led out of the system by means of valves so that no accumulation of carbon dioxide took place. The breathing took place through a trachea cannula.

The adrenalectomy in these acute experiments was performed simply by placing silk ligatures around the adrenal arteries and veins. After establishing a constant rate of pancreatic secretion the ligatures were tied. This was considered to be sufficient to prevent any discharge of the adrenal products into the circulation.

RESULTS AND CONCLUSIONS

Tables 1, 2 and 3 show the effect of adrenalectomy on the pancreatic secretion. In all instances the eclusion of the adrenals from their blood supply was followed by an immediate reduction in the pancreatic output. The secretion settled to a different level but then remained fairly constant during the next 30—50 minute observation period.

After establishing this control level the trachea cannula was connected to the gas-container. The time of anoxia ranged from 8 to 30 minutes.

In previous experiments (5) illustrated by the figure 1 with

TABLE 1

EFFECT OF ANOXIA ON THE PANCREATIC SECRETION: DOG 1

Control			
period, minutes	10^1	10^2	10^3
rate, cc/min.	0.25	0.25	0.25
Adrenalectomy			
period, minutes	10^1	10^2	10^3
rate, cc/min.	0.15	0.16	0.15
8 per cent O_2			
period, minutes	3^1	5^1	
rate, cc/min.	0.14	0.13	(stopped breathing)

[1] The secretin used in this work was prepared by the method of Krub (1948) and also kindly supplied by him.

TABLE 2

EFFECT OF ANOXIA ON THE PANCREATIC SECRETION: DOG 2

Control						
period, minutes	10^1	10^2	10^3	10^4		
rate, cc/min.	0.22	0.28	0.25	0.25		
8 per cent O_2						
period, minutes	5^1	5^2	10^2			
rate, cc/min.	0.40	0.19	0.09			
Normal air						
period, minutes	5^1	5^2	10^2	10^3	10^4	
rate, cc/min.	0.19	0.50	0.50	0.48		
Adrenalectomy						
period, minutes	5^1	5^2	10^2	10^3	10^4	10^5
rate, cc/min.	0.04	0.13	0.14	0.16	0.20	0.18
8 per cent O_2						
period, minutes	5^1	5^2	10^2			
rate, cc/min.	0.20	0.20	0.16			
Normal air						
period, minutes	10^1	10^2	10^3			
rate, cc/min.	0.11	0.25	0.22			
8 per cent O_2						
period, minutes	10^1	10^2				
rate, cc/min.	0.16	0.19				

TABLE 3

EFFECT OF ANOXIA ON PANCREATIC SECRETION: DOG 3

Control					
period, minutes	10^1	10^2	10^3	10^4	
rate, cc/min.	0.09	0.10	0.08	0.08	
Adrenalectomy					
period, minutes	5^1	5^2	10^2	10^3	10^4
rate, cc/min.	0.06	0.07	0.09	0.08	
8 per cent O_2					
period, minutes	5^1	5^2	10^2	10^3	
rate, cc/min.	0.10	0.12	0.18	0.20	
Normal air					
period, minutes	5^1	5^2	10^2		
rate, cc/min.	0.17	0.15	0.16		

intact dogs anoxia first caused in most cases an immediate increase in the pancreatic secretion. This effect was considered to be caused by hyperventilation and perhaps an attempt to maintain the normal

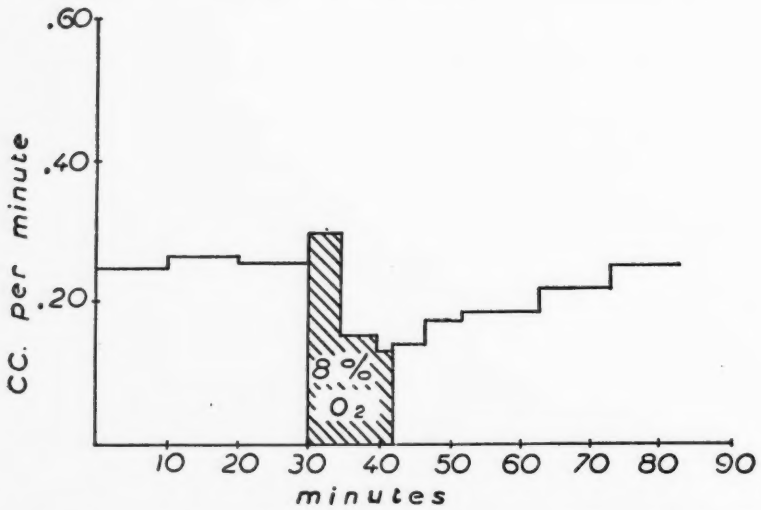

Fig. 1. — Effect of anoxia on the pancreatic secretion. The graph represents the mean rate of secretion of 13 dogs during a constant secretin-pancreozymin stimulation. The striated area presents the anoxia period. (K. Hartiala: Ann. Acad. Sci Fenn. A.V.F. 25. 1951).

acid-base balance in the organism under the conditions. In the present experiments this initial increase in the output was not present. It should be pointed out that the adrenalectomized animals showed very irregular breathing during the anoxia experiment: the respiration actually stopped in one case (dog 1). It was easily restored, however, by artificial respiration and allowing the animal to breathe normal air.

In this dog (table 1) the experiment had to be discontinued due to the irregular breathing. In this animal the pancreatic secretion showed a slight reduction during anoxia, this reduction, however, was not very great and did not show any tendency to further reduction as was the case in all previous experiments with intact dogs.

Table 2 reports the results obtained with dog 2. In this dog an anoxia procedure was performed before the ligation of the adrenal vessels. The usual change in the secretory pattern was present in this experiment. The behaviour of the pancreatic secretion during anoxia after the adrenalectomy was performed was quite different showing no greater reduction anymore.

The results with dog 3 (table 3) are similar to those in dog 2.

Anoxia caused no reduction in the pancreatic secretion in the adrenalectomised animal.

VAN LOO *et al.* (13) studying the pressor responses in anoxemia concluded that the adrenals come into play in conditions of anoxemia to help neutralize the ill effect of these states by raising the blood flow to the vital organs. The present observations lend some support to this claim. They also indicate that the anoxic suppression of the external pancreatic secretion is at least partially a consequence of this circulatory rearrangement regulated by the adrenal glands.

SUMMARY

Studies were made of the pancreatic secretion in dogs during anoxic anoxia after ligation of the adrenal bloods vessels. The anoxia was produced by allowing the animals to breathe an 8 per cent oxygen 92 per cent nitrogen mixture.

It was found that the secretory pattern of the pancreas during a constant and continous secretin-pancreozymin stimulus under the described conditions was not regularly affected by anoxia.

These results are in contrast to those obtained in animals with intact adrenals.

The present results seem to indicate that the anoxic suppression of the pancreatic secretion usually induced by anoxia is at least partially a consequence of adrenal activity and most likely a result of the increased adrenaline discharge under the conditions.

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FROM THE DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF HELSINKI

THE BENEFICIAL EFFECT OF ESTROGENS ON EXPERIMENTAL CINCHOPHEN ULCER

STUDIES ON ITS MECHANISM[1]

by

K. J. V. HARTIALA, A. KASSINEN and H. SUUTARINEN

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The relationship between sex hormones and ulcer disease has attracted a considerable amount of interest among both clinicians and physiologists. Sex hormones have been used for the treatment of peptic ulcer with claims of satisfactory results ((1, 2, 3, 8, 9, 12, 13). The possible mechanism of the claimed beneficial action exerted by sex hormones has so far remained unknown. The literature connected with the problem has recently been reviewed by Kirsner (7).

In a series of studies dealing with the production and treatment of the experiment cinchophen ulcer Nassio (11) reports that the administration of certain estrogens is capable to prevent the formation of ulcers in the majority of the cinchophen treated dogs. Previous studies have introduced experimental evidence which seem to indicate that the mechanism of cinchophen ulcer lies in the primary dysfunction of the duodenal (Brunner's) and pyloric glands (5, 6). Considering the hypothesis that the Brunner's secretion really has some causative bearing on the formation of cinchophen ulcer one might then expect that the estrogens should exert their action also on the duodenal glands.

In order to elucidate these relationships a series of experiments was conducted in this laboratory. In this work the secretory

[1] Aided by a grant from the Sigrid Jusélius Foundation.

function of the duodenal segment containing the Brunner's glands was studied during cinchophen toxication in both intact and estrogen treated dogs.

MATERIAL AND METHODS

Six mongrel female dogs were used in the experiments. The dogs were prepared with chronic duodenal flap type of pouches. Operative procedures have been described in previous papers (4, 5). The hourly output of duodenal juice was recorded both under basal and after-meal conditions. The daily observation period was 6 hours. The experimental procedures have also been described in the previous papers. Only the amount of juice was considered so that no chemical analyses on its constituents were performed.

Cinchophen was administered by feeding it through a stomach tube. The daily dose, 200 mg per kg of body weight, is known to be ulcerogenic in this animal.

The estrogen preparation was administered by intramuscular injections. The doses are reported in connection with the results. Only one type of estrogen preparation has been used so far. This synthetic product »Oestilben forte» (Orion, Helsinki) contains diaethyldioxistilben dipropionate 2.5 mg per ml.

RESULTS

The control secretory levels were established for each animal before each phase of the experimentation by daily observations over a period of 14—21 days.

In the first part of the work the effect of the estrogen preparation *per se* was studied. The results are listed in tables 1 and 2.

In table 1 it can be seen that the daily administration of 0.25 mg of the synthetic preparation on 6 days resulted in a moderate although statistically significant increase in the duodenal secretion in one dog (R). This effect was still present 3 weeks after the last injections were given. It is interesting to note that the increase was confined only to the after-meal secretion. The maximal output, 6.0 cc per hour, is more than about twice the maximal control value.

In the other dog treated similarily no changes in the duodenal secretion took place.

Similar studies with varying doses of the estrogenic prepara-

TABLE 1

EFFECT OF ESTROGENS (DIAETHYLDIOXISTILBEN; ORION: OESTILBEN) ON THE MEAN HOURLY OUTPUT OF DUODENAL GLANDS IN DOG. BASAL AND AFTER-MEAL SECRETION, CC/HOUR

	Control 14 Days		Hormone Treatm. 6 Days		Late Period 14 Days	
Dog R.						
Mean	2.3	2.55	2.5	3.35	2.4	4.2
St.d.	0.6	0.7	0.5	0.8	0.35	1.0
Range ..	1.9—2.3	1.3—3.2	1.8—3.3	1.9—4.4	2.1—2.9	2.8—6.0
Dog X.						
Mean	0.7	0.8	0.7	1.05	0.85	1.0
St.d.	0.4	0.3	0.25	0.5	0.2	0.35

TABLE 2

EFFECT OF ESTROGENS ON THE MEAN HOURLY OUTPUT OF DUODENAL GLANDS IN DOG. BASAL AND AFTER-MEAL SECRETION CC/HOUR,

	Dog A		Dog P		Dog R	
Control, 12 days. Mean	1.4	1.2	2.2	2.5	1.4	2.2
3×0,25 mg Oestilben, mean	1.5	1,5	—	—	1.7	2.3
After period, mean of 14 days	1.9	1.8	—	—	1.7	2.0
7×0,75 mg Oestilben, mean	2.3	2.0	2.4	2.6	1.6	1.9
After period, mean of 7 days ..	1.9	2.0	2.0	2.7	1.5	2.2
One month later, mean of 5 days	1.9	1.9	2.3	2.0	1.3	1.2

TABLE 3

EFFECT OF ESTROGENS ON THE MEAN HOURLY OUTPUT OF DUODENAL GLANDS IN DOGS TREATED WITH CINCHOPHEN. BASAL AND AFTER-MEAL SECRETION CC/HOUR

	Dog A		Dog P		Dog R		Dog E		Dog M	
Control, 14 days, mean ..	1.2	1.3	2.9	3,2	1.3	1.3	1.6	1.7	2.2	3.0
Cinchophen treatm. 11 days	1.7	1.7	1.6	2.7	1.7	1.4	1.6	1.3	2.7	2.7
2 last days, mean.....	1.1	1.6	2.0	1.7	1.6	1.4	0.8	0.6	1.1	1.2
After period, mean of 5 days	1.6	1.7	1.9	2.1	1.6	1.8	1.2	1.3	2.3	2.7

tion were thereafter performed. These were carried on three dogs. The results are given in table 2. The dogs were first given 0.25 mg for three days and thereafter 0.75 mg on 7 consequtive days. From the results it became evident that in general the duodenal secretion

was not effected by the estrogenic preparation with the exception of one dog (A) in which a doubtful increase could be noted.

The experiments were then continued with cinchophen. The drug was given to all dogs having previously received the estrogen treatment and in addition two new untreated dogs were taken in use. The effect of the cinchophen treatment is illustrated in table 3. From the previously estrogen-treated group (dogs A, P and R) cinchophen caused only in dog P a reduction in the duodenal secretion whereas no change was present in dogs A and R. It happens that A and R are the same dogs that had responded to the estrogen treatment with an increase of the duodenal secretion. In dogs E and M which had no previous estrogen treatment cinchophen caused a heavy reduction in the duodenal secretion similar to that obtained usually by this drug (5). The cinchophen treatment was stopped at the point where the duodenal secretion of dog E had reduced to some 30 per cent and in dog M to some 40 per cent of the control levels, this in order to save the animals for further experimentation.

After allowing the animals to recover from these experiments they were continued 6 weeks later. Now the previously intact dogs (E and M) as well as dog P received the estrogen treatment simultaneously with the cinchophen treatment. The former treatment was begun 10 days before the cinchophen treatment.

TABLE 4

EFFECT OF ESTROGENS ON THE MEAN HOURLY OUTPUT OF DUODENAL GLANDS IN DOGS TREATED WITH CINCHOPHEN. THE ASTERISK DENOTES THE DOGS TREATED WITH ESTROGENS. BASAL AND AFTER-MEAL SECRETION, CC/HOUR

	Dog A		Dog P*		Dog R		Dog E*		Dog M*	
Control, mean of 11 days	1.9	1.8	2.4	1.9	1.4	1.7	1.3	1.7	1.7	2.2
Cinchophen treatm. 11 days										
Total period. Mean	1.7	1.6	1.5	1.5	1.5	1.5	1.9	1.9	2.3	1.7
2 last days. Mean	1.6	1.6	1.4	1.0	0.9	0.7	1.8	2.6	3.5	2.4

The results are listed in table 4. From the estrogen untreated animals dog R responded now to cinchophen with a decrease of duodenal secretion whereas dog A still showed no reduction. Dog P in which previous experiments had shown no response tc estrogen treatment again showed a reduction during the cinchophen toxication. The most interesting results were, however, observed in

dogs E and M. In both dogs cinchophen failed to cause any reduction in the duodenal secretion. This secretion, as a matter of fact, was now slightly raised above the original control levels. It is interesting to add that at autopsy of these dogs immediately after this experiment there were no fresh ulcers in the stomach or duodenum. However, a large healed ulcer was present in the pyloric region in dog E, thisulcer obviously been produced during the first cinchophen treatment.

From these experiments it would appear that the estrogen treatment could prevent the reduction in the duodenal secretion which otherwise is inevitably caused by cinchophen. The mechanism of this favourable action on the duodenal glands deserves further studies. At the moment explanations of only speculative value can be offered.

SUMMARY

The effect on the duodenal secretion of a synthetic estrogen preparation (Oestilben forte, Orion, Helsinki) was studied in cinchophen treated dogs.

It appeared that cinchophen failed to cause the usual reduction in the duodenal secretion in 4 out of the 5 estrogen treated dogs.

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FROM THE DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF HELSINKI

STUDIES OF THE EFFECT OF HISTAMINE ON DUODENAL SECRETION[1]

by

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Histamine is a powerful stimulant of gastric secretion. It has also been used for the experimental production of peptic ulcer in various animals (1, 2, 3, 4, 10, 11, 12). The release of histamine following burns has been considered a causative factor in the development of peptic ulcer under these conditions. This possible role of histamine in this respect has been carefully reviewed by Ivy et al (9).

The organism is furnished with certain protective mechanisms affording it resistence against aggressive factors. The pyloric and duodenal (Brunner's) glands have been attributed the importance of protecting these particular regions against mechanical and chemical irritants (5, 6). Experimental evidence has also been advanced to support this claim (7, 8). In the series of studies which aim to elucidate the physiology and pharmacology of the duodenal glands it was considered warranted to include experiments on the effect of histamine on the secretion by these glands. To the writers knowledge, no similar studies have been previously performed.

MATERIAL AND METHODS

The experiments were carried out on 3 mongrel dogs prepared with chronic flap type duodenal pouches. The dogs weighed from 9 to 11 kilos. Both the experimental and the surgical procedures

[1] Aided by a grant from the Sigrid Jusélius Foundation.

applied in the work have been previously described in detail (7). Only the amount of the thick mucus output by the pouches was considered in the work.

Histamine — as histamine dihydrochloride — was administered by subcutaneous injections.

RESULTS

After establishing the control secretory level on each dog by daily 6 hour observations over a period of 3 weeks, 4 different kinds of experiments were performed as regards the dose of histamine used.

First, single 0.5 mg injections of the drug were given. In the 6 experiments performed in this way the administration of histamine was followed by a marked increase in the duodenal secretion in all but 1 experiment. The increase was present during the first two hours after the injection and ranged from 50 to 110 per cent over the control level.

Thereafter, the drug was given in 0.5 mg doses every 10 minutes over a period of 1 and 2 hours.

In these experiments no uniform response to the drug could be noted. This was due perhaps to the fact that histamine in these doses was found to produce nausea in the animals. In order to avoid this side-effect the experimentation was continued with 0.3 mg doses every 10 minutes over a period of 2 hours. The experiments were carried on for 9 consecutive week days.

The results are listed in table 1. When comparing the mean secretory values of the 9 day period with those of the control period it can be seen that only in one dog (A) was the secretion stimulated by histamine. No difference occurred in the other 2 dogs. The 9 day histamine period was divided into two parts in order to find out whether the secretory output differed in the earlier and later parts of the experiment.

As can be seen from the table, the secretion in 2 dogs was the same as during the control period. In the third dog the above-mentioned increase of the total period was due to the increased secretion during the latter part of the histamine period. During the first 3 days there was no increase. The highest secretion occurred during the 4^{th}, 5^{th} and 6^{th} days in this dog. After cessation of the

TABLE I

EFFECT ON DUODENAL SECRETION OF THE SUBCUTANEOUS ADMINISTRATION OF 0.3 MG DOSES OF HISTAMINE GIVEN EVERY 10 MINUTES OVER A PERIOD OF 2 HOURS. THE CONTROL VALUES REPRESENT THE MEAN RESULTS OF DAILY OBSERVATIONS OVER A PERIOD OF 3 WEEKS. HISTAMINE GIVEN DURING THE 2ND AND 3RD HOURS

Period	Dog A					Dog P					Dog R				
Control	cc of juice during the					cc of juice during the					cc of juice during the				
	1st	2nd	3rd	4th	5th hour	1st	2nd	3rd	4th	5th hour	1st	2nd	3rd	4th	5th hour
Mean	1.5	1.5	2.0	2.1	2.3	2.5	2.4	3.0	2.5	2.5	1.9	1.6	2.4	1.6	1.5
St.D.....	.4	.5	.4	1.2	1.4	.7	.9	1.0	.7	.6	.4	.4	.6	.6	.1
Histamine. Total															
Mean	2.6	2.7	3.1	2.8	2.1	2.8	2.9	2.9	2.2	2.3	1.7	1.5	2.0	1.8	1.8
St.D.....	.4	.3	.5	1.1	.5	.1	.7	.5	.1	.8	.2	.6	.7	.1	.3
First 3 days															
Mean	1.4	1.4	1.8	1.9	1.8	2.5	2.8	2.8	2.2	2.3	1.6	1.6	2.2	2.1	1.7
Last 6 days															
Mean	3.0	3.3	3.7	3.2	2.2	2.7	3.0	3.0	2.2	2.2	1.7	1.5	2.0	1.7	1.8
Control, 2 weeks later															
Mean	1.5	2.0	2.4	2.2	1.6	2.5	2.3	3.3	2.8	2.0	1.1	1.1	2.7	1.8	1.2

histamine administration the secretory output by the duodenum levelled down to the control values.

DISCUSSION

These experiments indicate that histamine in single doses tends to increase duodenal secretion. Whether this effect means a direct stimulation of the duodenal glands by histamine cannot be stated. It is known that the duodenal glands are under both nervous and humoral control (5, 6). The increased HCl-output of the stomach caused by histamine would set in action the humoral mechanism which is mediated through the duocrinin-mechanism previously demonstrated. We would be inclined to interpret the observed increase caused by histamine as a result of increased gastric secretion. If the effect is a direct one a more constant increase in the duodenal secretion after histamine might perhaps be expected. That the prolonged administration of the drug did not consistently increase this secretion seems to speak against a direct stimulating

action. From the gastric secretion we know e.g. that prolonged histamine administration causes a continuous abundant output from the fundic glands. From our previous knowledge, the duodenal glands are not easily exhausted hence the exhaustion of the glands does not explain the results. Studies made under condition in which the gastric secretion is eliminated should be able to prove our interpretation.

SUMMARY

The effect of varying doses of histamine on duodenal secretion was studied in dogs prepared with chronic duodenal pouches.

Single 0.5 mg injections of the drug resulted in an increased mucus secretion by the duodenal glands.

Prolonged administration of the drug in 0.3—0.5 mg doses given every 10 minutes over a period of 1 to 2 hours either had no effect on the secretion or was followed by an increased duodenal secretion.

The possible mechanism of the stimulating effect of histamine on duodenal secretion is discussed.

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